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PHYSIOLOGY

SENSITIZATION OF MECHANOCEPTORS BY ACETYLCHOLINE

I. T. Dzheliev and V. N. Chernigovskii

From the Laboratory of General Physiology (Head – Active Member of the AMN SSSR, V. N. Chernigovskii) Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR, V. N. Chernigovskii) AMN SSSR, Moscow, Received February 19, 1959

It has frequently been demonstrated that several chemical substances can not only stimulate particular chemoceptors, but may also sensitize or desensitize mechanoceptors [2,4]. A detailed review of this subject has been given by A. S. Paintal [3] in his paper at the Twentieth International Physiological Congress. However, there are no references to sensitization or desensitization of the intestinal mechanoceptors.

We have attempted to study the effect of acetylcholine on the mechanoceptors of the small intestine of cats.

METHOD

The cats were anesthetized with an intravenous injection of 20% urethane solution, and 35 acute experiments were carried out. The ileocecal portion of the intestine was separated, so that it retained only nervous connection with the rest of the body, and its vessels perfused with Ringer's solution. This particular part of the intestine was chosen because well marked reflex changes in arterial pressure and respiration can be elicited from the interoceptors of the ileocecal angle both by chemical and by mechanical stimuli. Solutions of $1:10^7$ – $1:10^3$ of acetylcholine and a 0.1% solution of atropine were used to act on the interoceptors. These substances were introduced into the perfusate through a syringe, the amount injected being usually 1 ml. Also, mechanical stimlation of the perfused section was applied by expanding it with air, whose pressure was measured in millimeters of mercury. Kymographic traces of respiration, pressure in the arterial artery, and of the heart beat (recorded through a membrane manometer) were also made. The rate of perfusion was determined by counting the perfusate drops electrically.

RESULTS

As a rule, mechanical stimulation of the perfused section produced a reflex elevation of the arterial pressure, and occasionally an increase in the rate and depth of respiration. Usually, a well shown effect was obtained simply by maintaining the pressure in the perfused portion at 60 mm, but sometimes it was necessary to raise it to 80 mm. As a rule, the reflex elevation of the arterial pressure was maintained during the whole period of mechanical stimulation. In a few experiments it returned to its initial level long before mechanical stimulation had ceased.

On introducing acetylcholine into the perfusate, the typical increase in arterial pressure and respiration rate was observed. We first attempted to find whether acetylcholine affects the nature or the extent of the reflex response to mechanical stimulation. For this purpose, experiments were carried out in which the perfused portion was stimulated mechanically before various concentrations of acetylcholine were intoduced into the perfusate. In the great majority of experiments, prior treatment with acetylcholine caused a marked change in the magnitude of the reflex response to stimulation of the mechanoceptors. The results were particularly clearly shown when the moderate or high concentrations of acetylcholine ($1:10^5$ – $1:10^4$) were used. In these experiments, after treatment with acetylcholine, the increase in arterial pressure was greater than the initial response.

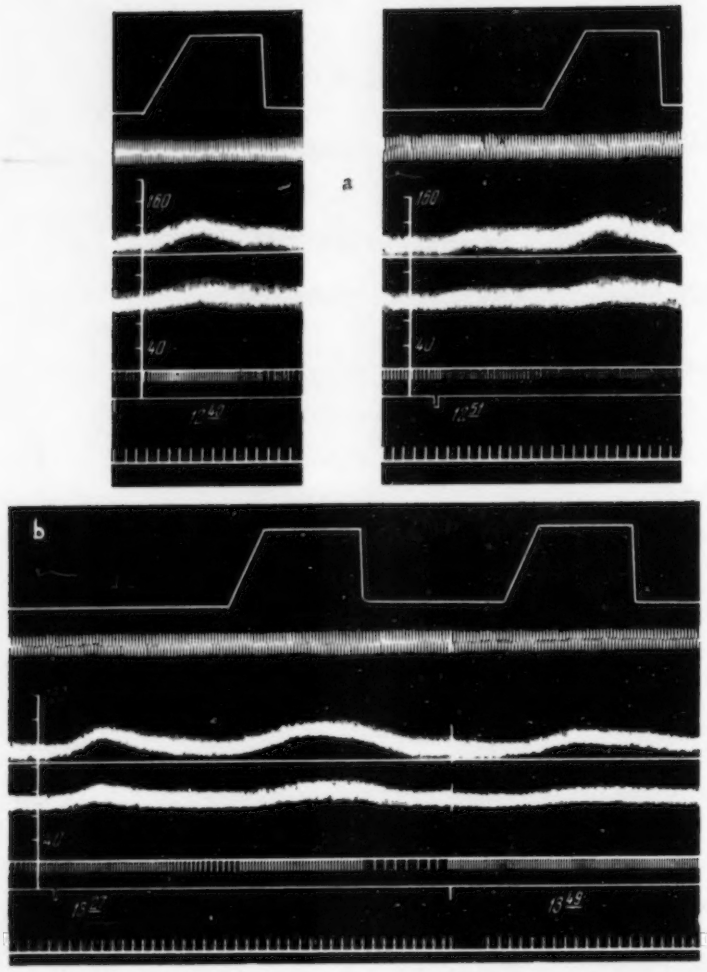

Fig. 1. Variation in the sensitivity of mechanoceptors of the ileocecal portion of the intestine after treatment with acetylcholine. a) No increase in sensitivity to mechanical stimuli with small doses of acetylcholine; b) effect occurs after introducing moderate or large doses of acetylcholine into the perfusate. Curves, from above downwards: strength of mechanical stimulus to perfused loop (in mm mercury), respiration, arterial pressure (in mm mercury), zero line, heart rate, perfusion rate in drops, stimulus marker, time marker (5 seconds).

In 10 experiments, threshold doses of acetylcholine $1:10^7 - 1:10^6$ gave no effect, but the higher concentrations were always effective (Fig. 1,a, b).

The increase in response to mechanical stimulation after treatment with acetylcholine usually developed after 10 minutes. During this period, the reflex remained at the normal level. In some experiments, even the single injection of acetylcholine was sufficient to maintain an increased reflex effect for a period of an hour or more.

If moderate or high concentrations ($1:10^5 - 1:10^3$) of acetylcholine were used, the effect did not always occur immediately. In 7 out of the 30 experiments, the first stimulus applied after giving the drug produced the normal response. Sometimes the reflex was even reduced. Only after the second or third stimulus had been applied, did the marked increase in size of the reflex occur (Fig. 2).

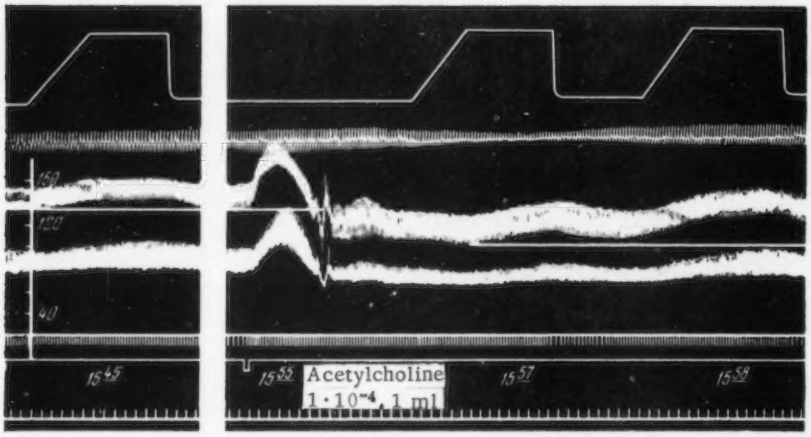

Fig. 2. Gradual increase in sensitivity to mechanical stimulation following moderate or large doses of acetylcholine. Curves as in Fig. 1.

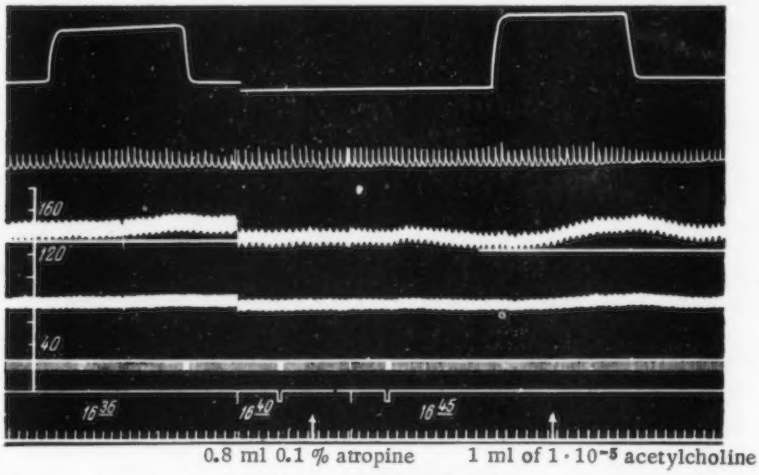

Fig. 3. Increase in the sensitivity of the perfused portion of the intestine to mechanical stimuli following previous treatment with acetylcholine (experiments with atropinized intestinal loop). Curves as in Fig. 1.

In these experiments, the increased sensitivity to mechanical stimuli usually developed over a period of 1-3 1/2 minutes after the first mechanical stimulus was applied. The fact that the effect was mediated by the acetylcholine can be seen from the experiments in which repetitive mechanical stimulation of the interoceptors without previous treatment with acetylcholine caused no increase in the reaction.

Quite frequently, the affect of acetylcholine was to change not only the extent but also the shape of the curve of the reflex response. Normally, after mechanical stimulation had ceased, the arterial pressure rapidly returned to its initial level, but when acetylcholine was given, an after-effect lasting 1.2-20 minutes occurred.

Finally, it must be pointed out that the reflex response to mechanical stimulation following the action of acetylcholine was present in full measure even when the mechanical stimulation of the receptors was delayed until after the direct action of acetylcholine on the arterial pressure and respiration rate had ceased.

When acetylcholine is introduced into the intestinal vessels, in addition to respiratory and circulatory

changes, there is also an increase in peristalsis, and therefore of intestinal tone. Therefore, the increased reflex response to mechanical stimulation which we observed to follow the injection of acetylcholine might be attributed to an increased tone of the intestinal wall, and not to sensitization of the mechanoceptors. To test this possibility, experiments were carriex out in which acetylcholine and mechanical stimulation were used after atropine had been introduced into the intestinal vessels. It was found that a certain dose of acetylcholine, usually 0.5-1 ml of 0.1% solution, still allowed an acetylcholine injection into the perfusate to provide reflex circulatory and respiratory changes, while at the same time peristalsis was completely arrested for at least 40 minutes. This dose of atropine in the perfusate as a rule caused no reflex change in the breathing or blood pressure; only occasionally was there a short, scarcely noticeable increase in arterial pressure.

Despite treatment with atropine, subsequent injection of acetylcholine into the perfusate increased the reflex circulatory and respiratory changes in response to mechanical stimulation, in just the same way as in the previous experiments (Fig. 3).

No response to mechanical stimulation was observed in cases when the injection of acetylcholine either had no effect on arterial pressure and respiration, or when such changes were very small.

The results obtained show that previous injections of acetylocholine into the perfusate increases the reflex response to a mechanical stimulation of this part of the intestine.

Concerning the mechanism, it appears that contact of acetylocholine with the interoceptors increases their sensitivity to mechanical stimulation. The experiments with atropine refute the idea that the observed effects are due to changes in the tone of the intestinal smooth muscle. Further, increasing tone of the wall of hollow organs does not increase but actually decreases the response to mechanical stimulation [1].

It follows, therefore, that the reason for the effect we have observed is due to the ability of acetylcholine to sensitize the intestinal mechanoceptors to their adequate stimulus.

SUMMARY

The experiments were performed on cats. The ileocecal portion of the intestine in which only the nervous connection with the rest of the body were left intact was perfused. Preliminary introduction of 1 ml of $1:10^5$ – $1:10^4$ acetylcholine into the perfusate causes an increase in the reflex changes in respiration and arterial pressure induced by stimulation of the mechanoceptors of the isolated portion of intestine.

The same effect was observed when the perfused area had been atropinized prior to giving the acetylcholine and stimulating mechanically.

The results show that acetylcholine has a sensitizing effect on mechanoceptors.

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* In Russian.

EFFECT OF LOCAL ACTION OF HEAT AND COLD ON ARTERIAL AND VENOUS PRESSURES, LYMPH FLOW AND RESPIRATION

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Presented by Active Member of the AMN SSSR, V. N. Chernigovskii, Received June 10, 1956

It is known that if the whole body is heated or cooled by a large amount, changes in the cardiovascular system, respiration, lymph flow, and lymph formation occur. Thus, V. V. Parin, A. P. Polosukhin, and V. N. Chernigovskii [6] and A. M. Blinova [2] have shown that thermal stimuli cause an increase in the output of the heart. G. A. Malov has shown that warmth applied locally will raise venous tone, and that cold lowers it. A. G. Zabolotskii [3] in studying variations of venous pressure in healthy and sick human subjects observed that venous pressure is raised by hot baths, and lowered by cold. M. L. Kokhanina [4], working on adult dogs, showed that both heat and cold applied locally increase lymph flow; in her opinion, lymph flow changes occur reflexly in response to stimulation of the cutaneous receptors. L. A. Bermzhanova [1] obtained similar results in puppies. Cold or hot stimuli applied to the outer ear or hind feet increased lymph flow in the first few days after birth.

The object of the present investigation was to determine how lymph flow is related to arterial and venous pressure, and respiration, as affected by local thermal stimuli.

METHOD

Forty-one acute experiments were carried out on adult dogs under morphine-pentothal anesthesia. For the heat stimulus, water at temperatures between 40° and 50° was used, while the cold stimulus was given by snow, or tap water at temperatures from +2 to 15°; it was applied in the region of the groin, belly, or back. The stimulation was continued for 1-3 minutes. One ml per kg of a 1% solution of inulin was given as an anticoagulant. The method for recording changes in arterial and venous pressure, lymph flow, and respiration have been described in detail in our previous article [3].

RESULTS

Application of heat to the groin, belly, and back gave the same results. In 17 of the 26 experiments, there was no change in arterial pressure, in 6 it was raised, and in 3 it fell. The venous pressure in the femoral vein rose, except in 2 experiments when it remained unchanged; in most experiments there was also an increase of venous pressure in the portal vein, but no change in the jugular vein. Respiration remained constant. Lymph flow varied as follows: in 21 experiments it was increased, in 4 it remained unchanged, and in 1 it was reduced.

The results of the experiment on February 15, 1952 are presented as typical of those obtained. Local heating of the belly to 50° for 2 minutes 15 seconds had no effect on arterial pressure or on that in the jugular vein; pressure in the femoral vein rose from 33 to 48 mm of water, and after 50 seconds' stimulation, to 50 mm. There was a marked increase in the lymph flow (Fig. 1).

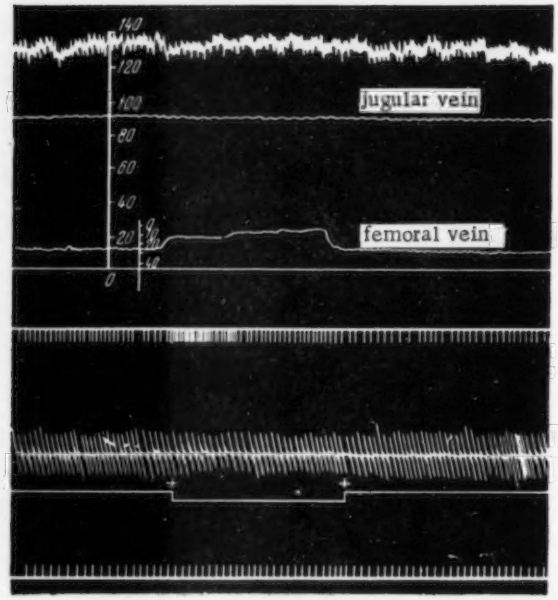

Fig. 1. Change in the reflexes in a 7 kg dog in an experiment performed on February 15, 1952 in which the belly was heated to +50° for 2 minutes 15 seconds. Curves, from above downwards: arterial pressure, two curves of venous pressure, zero line for arterial pressure, trace showing drops of lymph, respiration curve, stimulus marker, time marker (5 seconds).

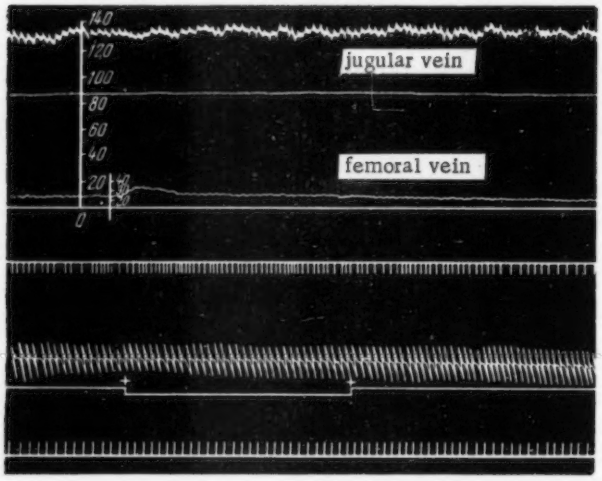

Fig. 2. Change in reflexes in a 10 kg male dog in an experiment performed on March 15, 1952 in which the inguinal region was cooled to -2° for 2 minutes. Curves as in Fig. 1.

With cold stimulation of the same regions, in 12 experiments there was no change in arterial pressure, in 1 it was raised, and in 2 it was reduced. The venous pressure changed in different ways: in the femoral vein, it was raised in 10 experiments, while in 3 there was no change; in the portal vein, it was raised in 1 experiment and unchanged in 3. In 10 of the experiments there was an increase in lymph flow, and in 5 no change occurred. Respiration did not vary.

As an illustration, the kymogram obtained in an experiment on March 15, 1952 may be considered (Fig. 2). The application of a cold compress to the inguinal region caused a marked increase in lymph flow in the theoracic lymph duct; it rose from 20 to 50 drops per 2 minutes, while the pressure in the femoral vein rose from 25 to 30 mm of water. There was no change in arterial pressure, pressure in the jugular vein, or respiration.

Reports have been published that lymph flow and venous pressure changes depend on the pumping action produced by the thorax and by muscular tension. In order to find the effect of these influences on lymph flow and venous pressure, we carried out experiments in which a bilateral pneumothorax was established, and artificial respiration maintained under deep anesthesia. When this was done, the same effects occurred as when normal breathing was maintained, but they were less well shown.

The results of the experiment allow us to conclude that both in experiments with natural breathing and when respiration was maintained artificially, changes in lymph flow and venous pressure in the femoral vein occurring in response to heating the skin of the groin, belly, and back occur reflexly and independently of changes in respiration of muscle tension. However, we do not deny that there is some relationship between lymph flow and various extracardial factors.

SUMMARY

Forty-one acute experiments were conducted on adult dogs. The inguinal region, abdomen, and back were heated to a temperature of 40-50°C or cooled to temperatures between -2° and 15°.

The observations were made while breathing was carried out naturally or by artificial respiration. It was found that local thermal stimulation provoked a reflex change of lymph flow and an increase in the venous pressure of the femoral and portal veins. However, lymph flow and venous pressure may depend to a certain extent upon various extra-cardial factors such as muscular tension, etc.

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*See C. B. Translation.

**In Russian.

RENAL SECRETION OF HYALURONIDASE IN DIFFERENT CLASSES OF VERTEBRATES

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Presented by Active Member of the AMN SSSR V. N. Chernigovskii; Received January 8, 1959

One of the most important adaptations involved in the transition from aquatic to terrestrial life is the evolution of an effective method of preventing water loss. An important factor in such a system is the antidiuretic hormone (ADH). It is found in the neurohypophysis of all vertebrate classes [5]. A. G. Ginetsinskii and his coworkers have recently shown that in the mammalian kidney, the action of the ADH is mediated by the enzyme system hyaluronidase – hyaluronic acid [2, 3].

From among the thousands of foreign and Russian papers dealing with hyaluronidase and hyaluronic acid, only a few report the presence of the enzyme in the kidney of various mammalian species [1, 6].

It seemed to us essential to discover whether hyaluronidase occurs in the kidney of all vertebrates.

METHOD AND RESULTS

The hyaluronidase was obtained from minced renal tissue, and the activity was determined by our own modification of the viscosimetric method [4]. The determinations were made on 25 species of animals (Table 1).

It can be seen from Table 1 that although there are quite big differences in activity within each class, considerable quantities of the enzyme were found in the kidneys of most animals. Those of the bullhead and pike contained such a large amount, that the tissue was scarcely less active than the commercial preparation "Ronidase". In the roach kidney, no enzyme was found at a dilution of 1:50. Similar variation occurred in birds: a high concentration of the enzyme was found in the kidneys of the wagtails and crossbills, while only small amounts were present in the gull and loon. It was difficult to find any relation between the activity of the enzyme and the ecological or physiological conditions of the species.

The substance which we extracted from vertebrate kidneys and which caused depolymerization of solutions of hyaluronic acid is certainly an enzyme. It was destroyed by boiling. Its activity fell logarithmically with the dilution.

To determine the part played by hyaluronidase in kidney function, we followed the excretion of the enzyme in water and osmotic diuresis. Bird urine, collected at low diuretic levels, always shows hyaluronidase activity, and this falls rapidly when urinary excretion rises after a water load (Fig. 1). The results obtained with osmotic diuresis also agree completely with those obtained by other authors in mammalian experiments [2]. When diuresis increases after intravenous injection of a hypertonic sodium chloride solution, the activity of the urine falls much less than when the urine secreted initially is diluted with distilled water (Fig. 2). In experiments where the water diuresis was arrested by injecting an extract of chick hypophysis, the concentration of hyaluronidase in the urine immediately rose.

TABLE 1

Activity of Renal Hyaluronidase in Different Vertebrates

Number, in order	Species	Temperature during test	Dilution	Activity (in arbitrary units)
1	River minnow, Lampetra fluviatilis	34°	1:50	12
2	The pike, Esox lucius	34°	1:50	40
3	Crucian carp, Carassius carassius	34°	1:50	11
4	The perch, Perca fluviatilis	34°	1:50	7
5	Bream, abramis brama	34°	1:50	7
6	The rudd, Scardinius erythrophthalmus	34°	1:50	5
7	The roach, Rutilus rutilus	34°	1:50	0
8	North Atlantic bullhead, Myoxocephalus scorpius	24°	1:50	57
9	The flounder, Limanda limanda	24°	1:50	32
10	The cod, Gadus morhua	24°	1:50	30
11	The White Sea herring, Clupea harengus	24°	1:50	32
12	The navaga , Eleginus navaga	24°	1:50	26
13	The frog, Rana temporaria	34°	1:50	5
14	The adder, Vipera berus	34°	1:50	5
15	The white wagtail, Motacilla alba	24°	1:50	36
16	The crossbill, Loxia pityopsittacus	24°	1:50	35
17	The oyster catcher, Haematopus ostralegus	24°	1:50	20
18	The black grouse, Lyrurus tetrix	24°	1:50	10
19	The redstart, Phoenicurus phoenicurus	24°	1:20	18
20	The tern, Sterna paradisaea	24°	1:20	12
21	The red throated loon, Gavia stellata	24°	1:20	10
22	The herring gull, Larus argentatus	24°	1:20	6
23	The chick, Gallus domesticus	34°	1:50	11
24	The common gull, Larus canus	24°	1:20	0
25	The white rat , Rattus novegicus albino	34°	1:50	20

Note: In cases where the activity of the hyaluronidase of test samples diluted 1 in 50 was low or absent, dilutions of 1:20 were used. Tests were made at 24° during an expedition to Ryazhkov Island, on the Kandalakshskii Reserve.

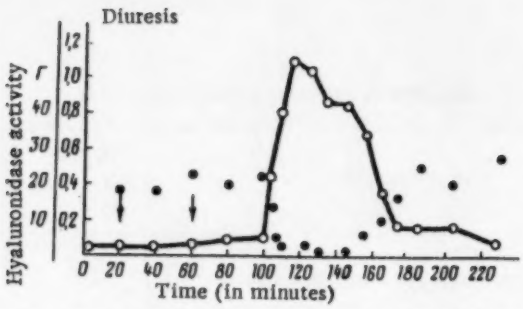

Fig. 1. Changes in hyaluronidase activity (in arbitary units) in chicken urine in water diuresis (in ml per minute). Points — hyaluronidase activity, curve — urine excreted, arrow indicates time at which 50 ml per kg of water were introduced into the intestine.

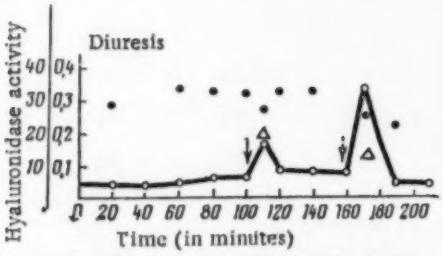

Fig. 2. Effect of osmotic diuresis on the excretion of hyaluronidase by the chick kidney. Triangle — activity of the initial urine sample, diluted with water to correspond to the degree of diuresis; ↓ — intravenous injection of 0.2 g per kg

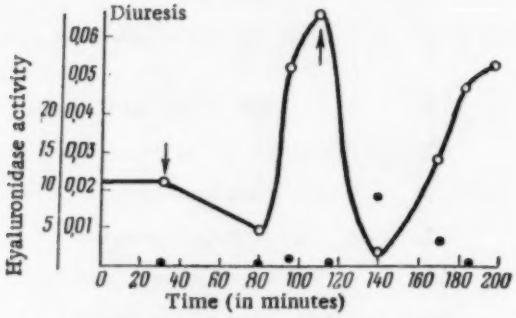

Fig. 3. Secretion of hyaluronidase by the kidney of the bog-turtle after injecting a turtle hypophyseal extract. ↓ — introduction of 50 ml per kg water into the intestine; ↑ — intravenous injection of turtle hypophysis extract. Curves as in Fig. 1.

The results obtained with osmotic diuresis also agree completely with those obtained by other authors in mammalian experiments [2]. When diuresis increases after intravenous injection of a hypertonic sodium chloride solution, the activity of the urine falls much less than when the urine secreted initially is diluted with distilled water (Fig. 2). In experiments where the water diuresis was arrested by injecting an extract of chick hypophysis, the concentration of hyaluronidase in the urine immediately rose.

From the results obtained it can be seen that just as in mammals, excretion of hyaluronidase by the renal parenchyma cells constitutes an essential link in the ADH mechanism.

Of the reptiles, we used the bog-turtle. No hyaluronidase was found in urine samples taken directly from the ureters in turtles during water diuresis, or in other samples taken from the bladder after cutting through the shell. However, if during the water diuresis the animal is injected with an extract of turtle hypophysis, the diuresis is inhibited, and hyaluronidase appears in the urine (Fig. 3). Some minutes later, the antidiuretic effect became weaker, urinary excretion increased, and the enzyme disappeared from the urine. Thus, it appears that in bog-turtles, ADH stimulates hyaluronidase secretion.

In frogs, whose bodies contain a lot of water, the rate of urinary excretion is high, and no hyaluronidase appears in the urine. If the animals are placed in a solution of sodium chloride, in order to cause a dehydration, diuresis is reduced, and some enzymatic activity is found in the urine (Table 2).

TABLE 2

Hyaluronidase Activity in Frog Urine

Number of test	Diuresis (in ml/hour per 100 g body weight)	Activity	Remarks
1	3,3	0	Summer frog taken from water
2	2,8	0	» » » »
3	1,37	0	Winter frog from water
4	1,22	0	» » » »
5	1,15	0	» » » »
6	1,06	0	» » » »
7	1,02	0	» » » »
8	1,05	2	Summer frog from NaCl solution
9	1,05	3	» » » » »
10	0,61	5	» » » » »

TABLE 3

Relationship of Hyaluronidase Activity in Pike Urine and Diuresis

Number of test	Diuresis (in ml/hour per 100g body weight)	Activity	Remarks
1	0,48	0	Pike from fresh water
2	0,32	0	» » » »
3	0,19	0	» » » »
4	0,15	0	Pike from NaCl solution
5	0,095	0	Pike from fresh water
6	0,085	3	Pike from NaCl solution
7	0,08	5	» » » »
8	0,06	7	» » » »
9	0,06	8	» » » »

From some of the sea fish, it was sometimes possible to obtain a sample of urine immediately after the animals were caught. This urine showed considerable activity; for instance in the bullhead it was 49 units, and in the cod 16 units. A systematic study of urinary hyaluronidase activity was made in the perch and pike. Studies were also made of the enzymatic activity of the urine of fish kept in fresh water, or dehydrated by immersion in various strengths of salt solution, which reduced diuresis. No hyaluronidase was found in perch taken from fresh water or from sodium chloride solutions. No enzyme was found in pike taken from fresh water. After being left for 3-5 hours in 1-1.3% NaCl solution, urinary excretion was reduced to a small fraction of its fresh water value. In most cases, hyaluronidase then appeared in the urine (Table 3), and the enzymatic activity was greater, the lower the diuresis. It is important to note, that according to V. F. Vasil'eva and M. M. Sokolova, under these conditions, the reduction of diuresis in the pike results not only from a reduced filtration rate, but also from an increase in facultative reabsorption, as can be deduced from the reduced excretion of the osmotically free water. Therefore, in some fish (pike), the reabsorption of water in the distal part of the tubule involves the excretion of hyaluronidase into urine.

Thus, hyaluronidase was found in representatives of all classes of vertebrates. It was shown that when diuresis was low, hyaluronidase activity was high, that in water diuresis the enzyme disappeared from the urine and that its activity was maintained during osmotic diuresis. We may conclude, therefore, that just as in mammals, facultative reabsorption of water in the tubules is associated with the secretion of hyaluronidase.

SUMMARY

At low diuresis rates, hyaluronidase was found in the kidneys and urine of representatives of all vertebrate classes. The enzyme was not present during water diuresis, but continued to be excreted during osmotic diuresis.

Its excretion into the urine was stimulated by the injection of hypophyseal extract. The results showed that facultative water reabsorption in the renal tubules of all vertebrates, including mammals, is connected with hyaluronidase secretion.

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**See English translation.

CHANGES IN FROG SKELETAL MUSCLE ACTION POTENTIALS FOLLOWING HYPOPHYSECTOMY AND ADRENALECTOMY

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Presented by Academician L. A. Orbeli; Received December 13, 1956

It has been shown previously [1, 2] that the voltage associated with the current of injury alters in response to hypophysectomy, adrenalectomy, or the injection of the corresponding hormones. The changes in the current of injury have been ascribed to disturbances of carbohydrate and phosphate metabolism resulting from the operations. The present work represents a study of the currents of injury as affected by removal of the hypophysis and adrenals.

METHOD.

The experiments were carried out on the sartorius muscle of autumn and winter frogs, the muscle being removed at a definite time after hypophysectomy or adrenalectomy. Muscles removed before either operation were used as controls.

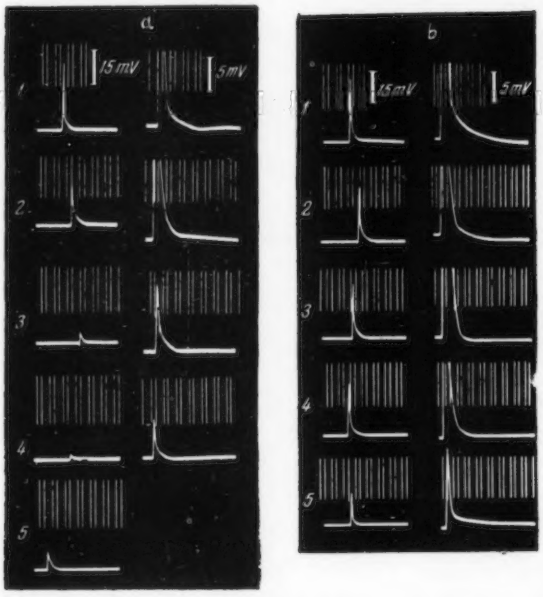

Fig. 1. Effect of adrenalectomy on action potentials. a) Experiment, b) control. 1) Initial recording; 2) after stimulating for 5 minutes; 3) after 10 minutes immersion in Ringer's solution; 4) after 60 minutes in Ringer; 5) after immersion for one day in Ringer; time marker 50 msec.

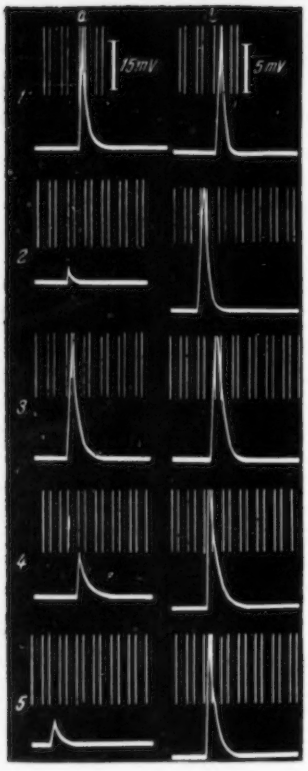

Fig. 2. Effect of hypophysectomy on action potentials. a) Experiment, b) control. 1) Initial recording; 2) after 3 minutes' stimulation; 3) after 10 minutes' immersion in Ringer; 4) after 60 minutes in Ringer; 5) after stimulating for 1 minute; time marker 50 msec.

For recording the action potential, the muscle was placed on a paraffin slab in a moist chamber. It was fixed in a somewhat extended condition in order to avoid distortion of the trace due to muscular contraction.

The action potentials were picked up by a unipolar electrode from the damaged and the undamaged part of the muscle through non-polarizable Zn – $ZnSO_4$ electrodes, and taken to a T-5 string electrocardiograph.

Electrical stimuli obtained from a neon discharge tube having a duration of 1 msec were applied at intervals of 1 second. The strength of the current was suprathreshold (the muscle contracted maximally).

The distance between the stimulating platinum electodes was 1-1.5 mm, while the stimulating and pickup electrodes were separated by 10 mm, the distance between the pickup electrodes being 5-10 mm.

Between the recording, the muscle was kept in Ringer solution. The method for removing the hypophysis and adrenals has been described in detail previously [1, 2]. In all, 30 experiments were carried out on each of the two groups.

RESULTS

Fig. 1 shows changes occurring in the action potential 5 days after removal of the adrenals, while Fig. 3 shows the corresponding change 3 days after hypophysectomy. It can be seen that after both operations, the traces obtained do not differ from those of the controls (see Fig. 1).

However, stimulating the muscle for 3-5 minutes at a frequency of 1 per second caused a marked reduction in the amplitude of the potential after hypophysectomy, while in control muscles there was no change (Fig. 2).

Placing the muscle in Ringer for 10 minutes after a 3 minute period of stimulation restores the shape and amplitude of the muscle potentials from a normal animal to their original value; in the adrenalectomized frog, not only is there no recovery, but a progressive decrease occurs.

If the muscle of either control group is left in Ringer for 1 hour, there is a marked reduction in the amplitude of the potentials obtained. There was no change in the controls.

These results show that both adrenalectomy and hypophysectomy cause marked alterations in the action potentials generated. Differences and resemblances can be found in the two operated groups; in both cases the amplitude of the potentials decreases when the muscle is left in Ringer, but the effect of stimulating for 3-5 minutes followed by an immersion in Ringer for 10 minutes is different in the two groups.

Because both the hypophysis and adrenals affect the level of carbohydrate and phosphorus metabolism, both directly, and indirectly through the other endocrine glands, the changes in muscle action potentials following removal of these glands is likely to be due to corresponding metabolic changes.

SUMMARY

Hypophysectomy and adrenalectomy caused changes in skeletal muscle action potentials. Stimulation for 3-5 minutes, or immersion for 60 minutes in Ringer, caused a reduction in the action potential amplitude.

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THE VAGUS NERVE AND THE CARDIO-GASTRIC REFLEX

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Presented by Active Member of the AMN SSSR V. V. Parin; Received December 31, 1958

We first demonstrated the reflex effect of the heart on the stomach in 1948 [1, 3]. It was shown that the mechanical vibratory cardiac stimulation not only affected the heart itself, but also produced a reflex effect on stomach movements. We confirmed the existence of this cardiogastric reflex by stimulating the heart with current from an induction coil [2].

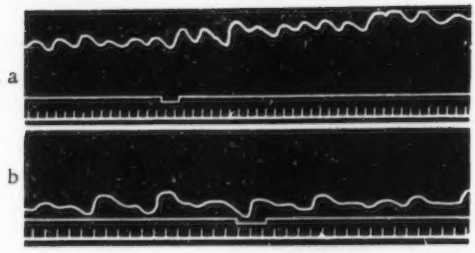
In the present study, we have made a first attempt to determine the nervous pathways involved in this reflex. We have shown previously [3], by selectively damaging different parts of the nervous system, that the reflex disappears only when the damage includes the medulla oblongata, which therefore appears to contain the reflex center. The nervous pathway of the reflex is still unknown. To determine it, we sectioned both vagi at their origin from the medulla.

METHOD

The experiments were carried out on bulbospinal frogs. The brain was sectioned immediately rostral to the medulla. After 30-40 minutes, after recovery from shock, the experiments were begun. The heart and stomach were exposed, and their movements recorded by levers on a drum for a period of 30-40 minutes. Two metal electrodes were then firmly fixed to the heart at a separation of 1 mm, and stimulation from an induction coil was applied. The primary of the coil was connected to a 6 volt storage battery; the voltage to the heart was suprathreshold,

Change in Gastric Motility Following Stimulation of the Heart with an Induction Coil

Type of change	Number of experiments	
	With vagi intact	Vagi sectioned
Increase in frequency of peristaltic waves	43	1
Increased height of waves	14	2
Reduced height of waves	–	1
Appearance of well-marked peristalsis, which did not occur before the experiment	4	–
No noticeable change in peristalsis	9	36
Total number of experiments.	70	40



Reflex change in gastric motility in the frog following cardiac stimulation before and after vagotomy. a) Increase in gastric contractions and increased gastric tone following stimulation of heart with current from an induction coil; b) after bilateral vagotomy; cardiac stimulation has no effect on gastric motility. Curves, from above downwards: gastric contractions, stimulus marker, time marker (30 seconds)

and the distance between the primary and secondary coils was 10-11 cm. The stimulus was maintained for 30-60 seconds, or more. The vagi were then cut, and after 30-40 minutes a further record of gastric movements was made for 20-30 minutes; this curve was used as a standard for comparison. Stimulation from the induction coil was once more applied, after which the stomach movements were again recorded for 20-30 minutes. In this way continuous recordings were made for a total period of 1 hour. At the end of the experiment, the average number of gastric contractions in equal 4 or 5 minute periods before and after cardiac stimulation were compared; measurements were also made of the amplitude of the contraction and of gastric tone.

RESULTS

Consistent results were obtained in 40 experiments in which the vagi were cut. They are shown in the Table. For comparison, results of experiments carried out previously [2], in which the vagi were not sectioned, are also given.

In 87% of the experiments in which the vagi were intact, stimulation of the heart with current from an induction coil caused a reflex increase in gastric motility, while after vagotomy, cardiac stimulation was without effect and in 90% of the experiments the gastric motility remained unchanged.

After cardiac stimulation in animals with sectioned vagi, there was, as a rule, no noticeable change in the tone of the gastric musculature; only in one single case was there a slight increase, while in one it was reduced; when the vagi were intact, there was an increased tone in 60% of the experiments.

We have therefore shown that the effect is conveyed from the heart to the stomach through the vagi, and, as has already been shown previously [3], through the medulla.

SUMMARY

In previous experiments on frogs and dogs, reflex effects on the stomach had been shown to follow mechanical or electrical stimulation of the heart.

In the present work, an attempt was made to trace the path of this reflex. In a frog, both vagus nerves were divided at the site of their exit from the brain. It was found that stimulation of the heart no longer affected the gastric movements.

The reflex also disappeared when the medulla was destroyed, while the vagi were left intact.

Therefore, the cardio-gastric reflex is effected through the vagi, and the medulla is the central link of the reflex arc.

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• In Russian.

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CUTANEOUS STIMULI AND THEIR RELATION TO SPINAL REFLEXES IN THE DOG

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Presented by Active Member of the AMN SSSR V. V. Parin; Received December 26, 1958

The problem of the restoration of function in a patient with a damaged or several spinal cord is one of the most difficult in the whole of neurology. In cases in which the cord has not been completely interrupted, a great deal of work has been done on remedial treatment, including passive and active exercises, massage, and baths [2, 3, 5, 7-9, 11, and others].

L. Guttmann [10] has claimed that even in patients with complete division of the cord, considerable compensation of function, and particularly of motor function, can be attained by special exercises. He attaches considerable importance to developing hypertrophy of the muscles of the upper part of the trunk, chiefly many of the back muscles. C. Long and E. Lawton [12] have described the muscular reorganization required for this purpose. By getting the patients to carry out exercises while in the vertical position, V. M. Ugryumov [6] and M. M. Krugly [1] obtained good results in cases of damage and even of interruption of the spinal cord. However, in spite of many attempts, the problem of the restoration of motor function in patients paralyzed as a result of spinal damage, has not yet passed beyond the experimental stage.

The present work reports results bearing on the part played by cutaneous stimuli in maintaining reflex activity in the distal part of the spinal cord in dogs after it had been completely divided, and the results may be relevant to the development of therapeutic methods.

METHOD AND RESULTS

In eight young adult dogs, the spinal cord was sectioned between the VI and VII thoracic vertebrae, and the animals were kept under observation for many years. They were divided into three groups. The first consisted of two dogs to whom no special cutaneous stimuli were applied, the second of two dogs in whom cutaneous stimulation was started 2 months after the operation, and the third of four dogs to whom stimulation was applied 2 weeks after the operation.

The cutaneous stimulation consisted of systematic use of massage, and also of scratching and pressure on certain of the paralyzed parts so as to elicit various spinal reflexes. In many cases, the mechanical stimulation was combined with electrical excitation applied through the skin of the foot. The stimulus was always suprathreshold, and caused a local spinal reflex. Three dogs of the last group were given massage and passive exercises daily. The living conditions and quarters were the same for all animals.

The reflexes studied were the extensor thrust, knee jerk, and flexor, scratch, and stepping reflexes; atypical motor reactions were also stimulated by stimulating fields outside those normally associated with a particular reflex. Such reactions included movement of the paw in response to electrical stimulation of the skin of the tail, tail movements evoked by electrical stimulation of the skin of the foot, etc. [4]. In all the spinal dogs, shortly after the operation and before the application of cutaneous stimulation, the flexor and knee jerk reflexes were weak; no atypical motor reactions were observed (Fig. 1).

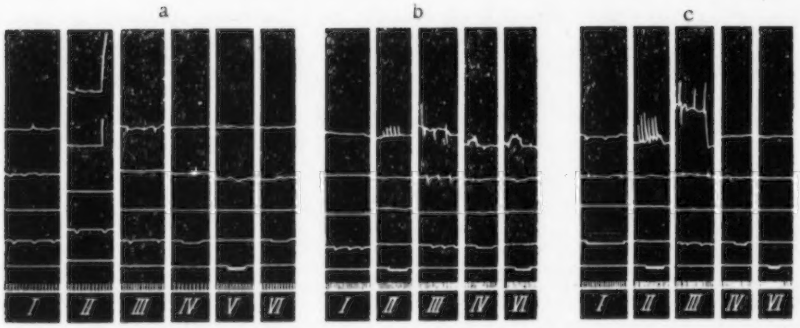

Fig. 1. Motor reflexes in spinal dogs 10 days after operation (before cutaneous stimulation). a) dogs from Group 1; b) dogs from Group 2; c) dogs from Group 3; I) extensor thrust; II) flexor reflex; III) knee jerk; IV, V) scratch reflex; VI) stepping reflex. Curves, from above downwards: movement of right foot; movement of left foot; tail movement; stimulus marker; time (1 second).

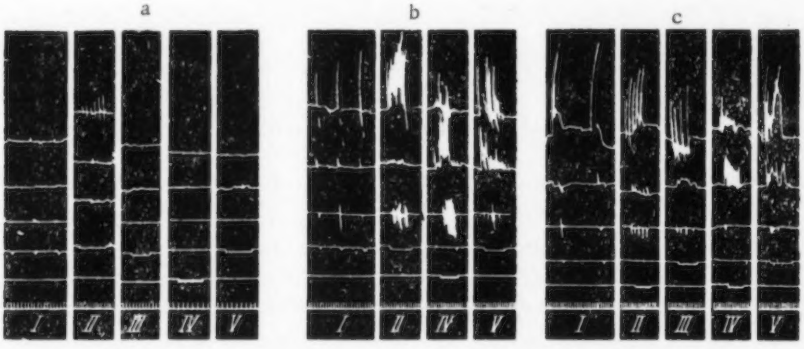

Fig. 2. Reflexes in spinal dogs with and without cutaneous stimulation. I) extensor; II) flexor reflex; III and IV) scratching reflex; V) stepping reflex. Other indications as in Fig. 1.

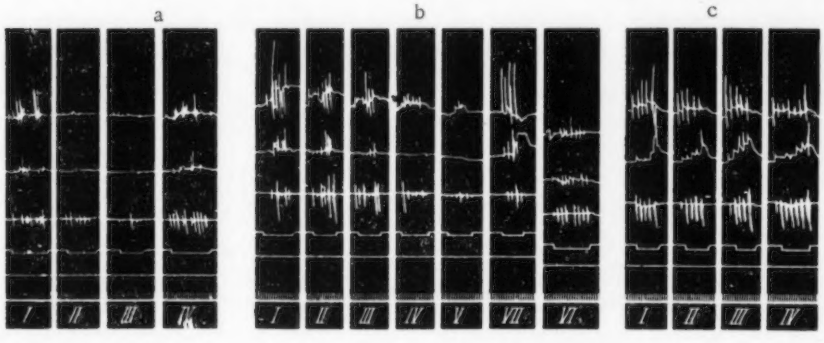

Fig. 3. Degree of stability of the atypical reaction in dogs of different groups. I) V) response to rhythmical stimulation of skin of tail; IVa and VI) response after a 10-minute rest; VII) response to stimulation of a second skin area in tail. Other indications as in Fig. 1.

In dogs of Group 1, (no special stimulation), there was some slight increase of the reflexes 1-2 months after the operation; subsequently they again became weaker, and after 6-7 months were at the original level (Fig. 2,a).

One year after the operation, just as before, only very limited reflexes could be evoked. Besides the flexor and knee jerk reflexes, in one a weak extensor thrust was developed, and in another the scratch reflex was present. In these animals there was considerable atrophy of the muscles of the hind limbs, and there was a deterioration of the joints. The hind limbs took very little part in walking, and usually dragged behind.

After 6-7 months, dogs of Group 2 who were given stimulation after 2 months, as a rule developed numerous well-marked but somewhat variable reflexes as well as an atypical tail reaction (Fig. 2, b). The condition of their hind limbs and method of movement was somewhat better than in those of the first group: although they crawled, the hing legs did not always drag, but took an active part in the movement.

The effect of cutaneous stimulation,begun immediately after the operation,was still more striking in the animals of the third group: after only 3 weeks from the time of the operation, good spinal reflexes developed, and even the atypical reaction of the paws in response to tail stimulation occurred (Fig. 2, c). Subsequently they moved better than dogs of the other two groups, because some of them developed a good extensor tone: they were able to stand, using their hind legs as a support, and even take a few steps without falling.

In order to obtain more detailed information about the functional condition of the caudal end of the cord as affecting the libility of nervous processes, we carried out tests to find whether the movement of the paw in response to tail stimulation occurred. To evoke this reaction, single shocks were applied simultaneously to the end of the tail and to the skin of the foot. The duration of each pulse was 100 msec, and the interval between them 2 seconds. A stimulus strength of 1.2-1.5 times threshold was chosen, and this caused a weak local reaction of tail and foot. The experiment was continued, and from 100 to 500 paired stimuli were applied. Dogs in the first group were first given the stimuli one year after the operation, the second group after two years, and the third group after 2 weeks. Before giving this treatment, none of the animals showed the atypical reaction.

The results obtained in group 1 were different from those in groups 2 and 3. Thus, in 4 animals of group 3, the atypical reaction of the paw appeared in the first experiment, although none of the animals had been giving massage or exercises. After a few repetitions, the reaction became strong and stable. Similar results were obtained in group 2. Thus, one animal was given 500 and another 600 stimuli in the second experiment, after which the atypical paw reaction was developed. However, at first the reaction was not stable, and did not become so until after the application of 3000 stimuli in one animal, and 5000 in the other; after this, it was maintained even after a 3-month interval.

In the first group, the atypical reaction of the foot to tail stimulation also appeared, although it was necessary to apply more stimuli than in group 2. However, the subsequent application of stimuli did not reinforce the reaction, and, on the contrary, it disappeared and did not return later, in spite of the fact that in 16-20 experiments the animal received 5000 double stimuli. Very little change in the other reflexes occurred through application of these stimuli. In one animal it was sometimes possible to induce an extensor thrust as well as the weak flexor reflex and knee jerk, and in another the scratching reflex could be evoked.

After carrying out these experiments on the dogs in group 1, a course of massage and passive exercise lasting almost 4 months was given, but the atypical reaction never appeared. There was scarcely any improvement either in the extensor thrust, scratching or stepping reflexes, despite a marked improvement in the condition of the muscles and joints of the hind limbs. Thus, cutaneous stimulation applied 1-1 1/2 years after the operation produces no essential change in the caudal section of the divided cord.

Of the other dogs, experiments with systematic massage, passive exercises, and electrical stimulation showed that quite early (10-14 days after the operation) the reflex activity of the caudal portion of the spinal cord returned rapidly. It appeared that systematic training undertaken shortly after the operation and designed to bombard the cord with afferent impulses, prevents the caudal portion from dystrophy and from the secondary effects resulting from it.

To test this hypothesis, we carried out experiments in which the nervous processes in the caudal portion were exercised to exhaustion. The experiments were performed as follows. During the period of active reflex activity, a voltage of 1.5 times threshold was applied to the skin, and the induced response of tail and paw was

recorded. The stimuli were given in groups at 1-minute intervals until the response began to fall off. The stimulation was then stopped, and renewed after a 10-minute interval. In the dogs of group 1, the atypical reaction ceased, even in the second group, the tail reaction was weakened, while in the case of the third group it almost disappeared. After a 10 minute interval, both reactions returned (Fig. 3,a).

In dogs of group 2, repetitive stimulation also caused an exhaustion of the atypical reaction as well as some weakening of the tail response, but for the latter it was necessary to repeat the stimulation 5-6 times. A control experiment to show that there was no fatigue in the muscle when the response failed was to stimulate another portion of the skin of the tail, which induced a well-marked reflex response (Fig. 3, b). It was not possible to observe any weakening of the reflexes in dogs of group 3: they were stable and well developed (Fig. 3, c). Consequently, the inability of dogs not receiving special stimulation to develop reflexes depends to some extent on the fact that such reflexes are easily exhausted.

The experiments demonstrated a functional difference between the caudal ends of the cords of stimulated and unstimulated animals. In the one case, it was possible to elicit spinal reflexes and even to develop new ones; in the second case, dystrophic processes resulting from inactivity caused almost complete loss of reflexes, which in turn affected the condition of the muscles and joints as well as the animal's movements.

The time elapsing between operation and treatment was of the greatest importance. When the time was short, the reflexes were rapidly activated, developing as the spinal shock wore off and remaining active for a long time. It appeared that having been freed from the influence of the higher centers, the spinal cord did not lose the ability to develop rapidly various kinds of reflexes, and that this ability, which was maintained by special stimulation, was retained and even showed some further development.

SUMMARY

Observations were made on 8 adult dogs in which the spinal cord was divided between the VI and VII thoracic vertebrae. Massage, scratching, and electrical stimulation were used to prevent dystrophy in the caudal portion of the cord. Two dogs were not stimulated, in two treatment was started after 2 months, while in the remainder the special stimulation treatment was started 2 weeks after the operation.

The activity and stability of various spinal cord reflexes and the ability to form atypical reactions served as the indices of the functional condition of the distal portion. The results of the experiments showed that the stimulation, especially when begun soon after the operation, was effective in arousing various spinal cord reflexes, even to the extent of elaborating new ones.

When no special stimulation was given, after only a few months, there was an almost complete loss of reflexes with inevitable deterioration of the condition of the muscles and joints and of locomotion.

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THE EFFECT ON ERYTHROPOIESIS OF SERUM FROM HEALTHY AND ANEMIC SUBJECTS

COMMUNICATION 1. CHANGES IN THE BLOOD AND BONE MARROW

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Until the last few years, insufficient attention has been paid to the erythropoietins (hemopoietins) as factors controlling erythropoiesis. However, between 1951-1952 and the present, increasing importance is being attached to those substances on account of their theoretical and physical importance. The conception of erythropoetins in the serum was first advanced in 1906, when Carnot and Deflandre found that serum from animals rendered anemic by bleeding acquires the property of increasing the erythrocyte count and hemoglobin in rabbits into which it was injected. This result led the authors to suppose that the regeneration of the blood is due to the increased formation of a special hemopoietic stimulator which they called hemopoietin. According to them, this substance is present, but in smaller amounts, in healthy animals.

Later Zhibelli showed that the hemopoietic activity of serum appears when the animals are rendered anemic by means of phenylhydrazine. Similar properties are developed in serum in hypoxia [5, 9].

Ya. G. Uzhanskii [2] has made a detailed study of hemopoietic substances occurring in the serum after bleeding. M. G. Kakhetelidze [1] used an original method of tissue culture to demonstrate the erythropoietic activity of normal plasma and its change in different pathological condtions.

Many authors [3, 7, 8, 10] have attempted to find erythropoietins in the serum of patients with different kinds of anemia, but most of them obtained positive results only in the posthemorrhagic and hemolytic anemias.

The most extensive and detailed study of the different hemopoietins has been made by Japanese workers. According to Komyia and his coworkers [7], they are formed in the liver and spleen. Their formation and entry into the blood stream is controlled by the splanchnic and vagus nerves. Once in the blood stream, they act directly on the bone marrow.

In spite of the large amount of work, much of which has been done abroad, until now neither the nature, mode of action, origin, or regulation of the secretion of hemopoietins has yet been established, and very little is known of the frequency of their appearence in clinical forms of anemia.

METHOD

A sample of 10-15 ml of blood was obtained from a vein, and after clotting it was centrifuged at 3000 rev/minute for 10 minutes. Then the serum was sucked off, and 3 ml injected simultaneously into each of two healthy rabbits; during the two previous weeks, the erythrocyte count, hemoglobin, and number of reticulocytes had been determined, and the condition of the bone marrow studied. With the exception of 3 animals, variation in the number of reticulocytes was not more than 1%. After the injection of serum, the blood was examined daily for 7 days, and then once weekly for the next month. The blood samples were taken from an ear vein. The bone

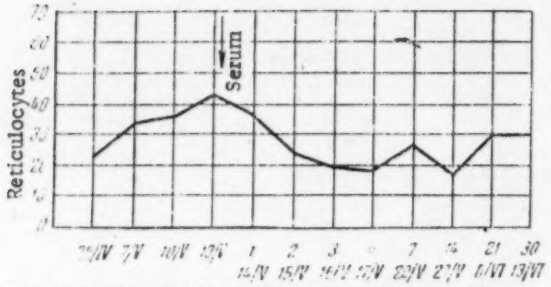

Fig. 1. Change in the erythrocyte count in a rabbit following injection of healthy human serum.

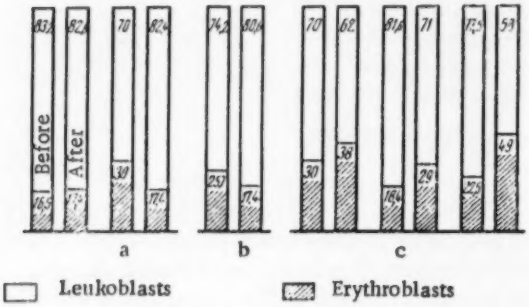

Fig. 2. Change in the ratio of erythroblasts to leukoblasts in the bone marrow (in percentage) in rabbits after injecting serum from healthy and anemic patients. a) Serum from healthy subjects; b) from patients, which does not induce reticulocytosis; c) serum which does induce reticulocytosis.

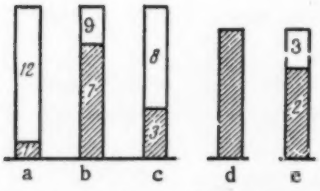

Fig. 3. Incidence of reticulocytogenic properities of the sera of healthy subjects and anemic patients. a) healthy subjects; b) B_{12}-deficient anemic patients; c) iron deficiency anemia; d) acute posthemorrhagic anemia; e) hypoplastic anemia; 1, 7, 3, 2) number of cases when serum caused no reticulocytosis; 12, 9, 8, 3) number of cases when the serum did cause reticulocytosis (numbers over the columns represent total number of subjects investigated).

marrow sample was taken by puncture of the epiphysis of femoral bone 4-5 days after injecting the serum.

In all, the serum was examined in 14 healthy subjects and in 29 patients with different kinds of anemia. Seventy-six rabbits were used and 31 injections given, of serum from 14 healthy human subjects.

RESULTS

The serum of only one of the members of the control group caused an increase in the number of reticulocytes, but in all the remaining cases, the variation in the reticular count was small (Fig. 1).

There was also very little change in the bone marrow after the injection, and the ratio of leukoblasts to erythroblasts remained unaltered (Fig. 2, a).

In no single case was any increase in the hemoglobin or erythrocyte count observed.

The absence of any reticulocytosis following the injection of healthy human serum into rabbits does not constitute a final proof of the absence of any erythropoietic stimulators in it, since the introduction of a foreign serum might by itself mask the action of small amounts of hemopoietin. That this is so is clear from the fact that injection of 3 ml of serum from 1 healthy rabbit into another, on two occasions caused an increase in the number of reticulocytes of 1 1/2-2 times.

Just as when healthy serum was injected, in no single case was there any increase in the hemoglobin or erythrocyte count after injecting anemic serum. At the same time, unlike healthy serum, that from the anemic patients much more frequently caused a marked reticulocytosis (Fig. 3).

The serum from 3 patients with severe posthemorrhagic anemia caused an average increase in the number of reticulocytes of 260% (increase from 2 to 5.2%); the reticulocytosis lasted for 3-4 days. The serum of 7 of the 9 B_{12}-deficient anemic patients caused an increase in the number of reticulocytes. Even by the 1-2nd day after the injection, a reticulocytosis developed and was maintained until the 5th day, representing an average increase of 236% above the original level (increase from 2.2 to 5.2%; Fig. 4). On no occasion did an injection from the other 2 patients cause any increase in the number of reticulocytes. The serum of only 3 of the 8 patients with gastrogenic iron deficiency anemia induced reticulocytosis, and this developed during the first few days after the injection, where the increase was 261% above the initial number of cells (increase from 2.1 to 5.6%). The serum of the remaining 5 patients had no such effect.

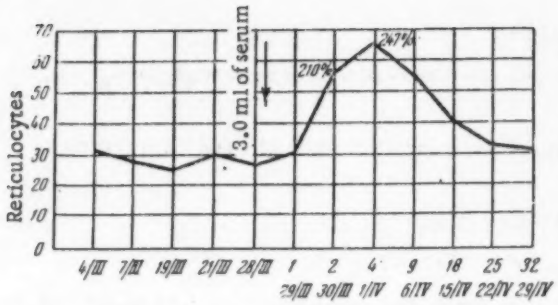

Fig. 4. Change in the number of reticulocytes after injecting serum from a patient with pernicious anemia at the height of the anemia.

The serum from one individual from the group with hypoplastic anemia, who died from this condition, caused not the slightest increase in the number of reticulocytes when injected into rabbits. Nevertheless, the serum from 2 other patients in whom there was a remission, caused an increase from 2.2 to 6.4%, or 287% above the initial level. This reticulocytosis developed on the 5-7th day after the injection, i.e. somewhat later than in the previous cases, and lasted for 3-4 days.

Injection of serum from a patient with a hereditary hemolytic anemia caused an increase in reticulocytes of 350% above the initial level (from 2 to 6 and 7%) in two rabbits into which it was injected. Serum from patients with various secondary anemias also increased the reticulocyte count, while sera from a patient with polycythemia vera and from 2 patients suffering from splenomegaly and cirrhosis of the liver caused no change in the reticulocyte number.

The reaction of the bone marrow to the serum injection was similar to that of the peripheral blood, i.e. the increase in the number of reticulocytes in the latter was accompanied by a reticulocytosis and an increased production of erythroblasts with a shift towards the polychromatophils in the bone marrow (see Fig. 2).

The variable condition of the patients and the fact that only the small number of 29 of them were available made it impossible for us to reach a final conclusion as to the result to be obtained with any particular kind of anemia; nevertheless, the impression was gained that the power to induce reticulocytosis in animals is present in the serum of patients with acute posthemorrhagic anemia (in agreement with published results); the same effect is still better shown in the serum of patients with B_{12}-deficiency anemia, though this result does not agree with that of Komyia [7]. At the same time, the reticulocytogenic effect is not typical for the sera of patients with iron deficiency anemia (see Fig. 3).

We were not able to observe any relationship between the reticulocytogenic effect of the serum and the stage of the desease, degree of the anemia, or number of reticulocytes.

Thus, injection of serum from patients with different kinds of anemia into healthy rabbits frequently caused an increase in the number of reticulocytes; the effect was well shown and appeared to be genuine, since it was accompanied by a corresponding reticulocytosis and erythroblastosis in the bone marrow. Since healthy human serum does not possess this property, it must be supposed that a large amount of some substance which stimulates erythropoiesis is formed in the blood of these patients. It remains to inquire whether these substances are genuine stimulators, or whether they lead to an increased erythropoiesis by bringing about some previous hemolysis; this view seems to us all the more likely because in no case did we observe a polycythemia vera. To find the answer to this and to many other problems concerning the mode of action of the erythropoietins, and their chemical structure, we have carried out further studies including experiments on pigment metabolism, the presence of hemolysins, and the significance of the globulin fraction, etc; the results will form the subject of a further paper.

SUMMARY

A subcutaneous injection of 3 ml of serum of patients suffering from acute posthemorrhagic anemia and from pernicious anemia provokes an increase in the number of reticulocytes in healthy rabbits. Not only is there a peripheral reticulocytosis, but there is also an increase in the number of reticulocytes and erythroblasts in the bone marrow.

The serum of some patients suffering from iron deficiency anemia possesses the same property, but the effect occurs less regularly than in the groups mentioned previously.

Some of the healthy persons has no effect on the number of reticulocytes. Possibly there is an increased production of erythropoetins in some forms of anemia.

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PERMEABILITY CHANGES IN VESSELS FOLLOWING BLOOD LOSS

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Most workers have studied permeability change in vessels either very soon after their occurrence, or else after severe hypoxemia; in clinical practice, hypoxic conditions, though not severe, are frequently prolonged, so that naturally, it is not possible to apply results obtained from acute cases to these conditions. Thus, for instance, it is generally accepted that a severe hypoxia increases vessel permeability, but that moderate hypoxia has no such effect. Little is known either about permeability changes occurring at different times after hemorrhage, or after other kinds of hypoxia.

E. Landis [10, 11] has shown that washing out the mesenteric vessels with Ringer's solution containing no oxygen causes a marked increase in their permeability; a similar change is induced by a three minute period of ischemia, caused by ligaturing the arteries. G. Saslow [14] perfused frog vessels, and observed an edema which could be eliminated by adding erythrocytes to the perfusate. F. Maurer [13], S. Drinker and M. Field [7], M. Field, A. Koranyi and G. Szabo [8] found an increase in capillary permeability and in lymph formation on adding hemoglobin and oxygen to the blood, and found the pulmonary vessels particularly sensitive to hypoxemia [17].

A relation between hypoxemia and a tendency to edema has also been observed clinically [6, 16]; on the other hand, when oxygen is administered, a reduction in the flow of fluid from the capillaries into the tissues has been observed [1].

Many authors doubt whether moderate oxygen lack causes any increase in blood vessel permeability [9, 12, 15]. G. Sh. Tairov [4, 5], has shown in animals and in blood donors that after bleeding, for several hours there was a reduction in peripheral circulation and an absorption of substances of low molecular weight from the skin in spite of the fact that the volume of the circulating blood and the blood pressure had regained their initial values some time previously.

The object of the present work has been to find the effect of moderate blood loss on general blood vessel permeability at later periods.

METHOD

The experiments were carried out on 22 rabbits and 14 dogs, who were divided into 4 groups according to the amount of blood lost (Table 1). Before the experiment, the animals were kept for 3 days under constant living and feeding conditions. The hemoglobin was then determined and a red cell count made. The animals were bled by withdrawing blood from the heart through a syringe; the total amount of blood taken was equal to 1/13 of the body weight. On the following day the same determinations were repeated, after which the total permeability of the blood vessels was determined. According to L. A. Oivin and his coworkers [2] a good index of this quantity is the rate at which substances of low molecular weight pass through the vessel walls. We therefore used a radioactive isotope of phosphorus in the form of $Na_2HP^{32}O_4$; 4 μ Cu per kg was injected into the jugular vein. After 5, 30, and 60 minutes, blood samples were withdrawn to determine its activity; for this purpose,

TABLE 1

Distribution of Animals According to Amount of Blood Lost

Group	Number of animals			Amount of blood lost (in %)
	rabbits	dogs	total	
1-	10	5	15	Control
2-	4	3	7	5
3-	4	3	7	10
4-	4	3	7	15

TABLE 2

Change in the Erythrocyte Count and Hemoglobin Content in Rabbits and Dogs After Bleeding

Group	Conditions of test	Average indices			
		rabbits		dogs	
		hemoglobin	erythrocytes (in thousands per mm^3)	hemoglobin (in Sahli units)	erythrocytes (in thousands per mm^3)
2-	Before bleeding	80	4 480	92	4 996
	After "	70	3 760	91	4 230
3-	Before bleeding	79	4 450	93	4 983
	After "	68	3 612	78	3 570
4-	Before bleeding	80	4 527	95	5 153
	After "	68	3 492	77	3 526

TABLE 3

Reduction in Radioactivity of Blood (in impulses per minute)

Group	Rabbits			Dogs		
	5 minutes	30 minutes	60 minutes	5 minutes	30 minutes	60 minutes
1	112	82	54	106	93	88
2-	97	80	57	65	56	53
3-	78	66	41	64	53	50
4-	69	56	53	66	52	45

0.03 ml of blood was transferred onto a standard sized filter paper, and after drying, the activity of the blood sample was determined on a type B instrument using an MS-4 electronic counter. The number of impulses in excess of the background level per minute was counted; the average of three counts was taken.

RESULTS

The results of all investigations are given in Tables 2 and 3.

In general, the reduction in the radioactivity of the phosphorus in the blood, both in rabbits and in dogs, corresponded to the amount of blood lost, the relationship being quantitative. It is especially in the first few minutes, when the concentration of the isotopes in the blood is highest, that there is the greatest loss of isotopes from the blood stream, and therefore that the permeability of the vessels to substances of low molecular weight is greatest. It seems to us that this phenomenon must be an adaptation to hypoxia. It is known that in oxygen lack, many compensatory processes develop which improve the absorption of oxygen by the tissues. Although the peripheral vessels are temporarily constricted, those of organs such as the heart, brain, and probably the lungs and other organs dilate, so that the increased permeability of the membrane of the vessel, occurring reflexly, eliminates the tissue hypoxia following a moderate blood loss [3].

The immediate reason for the increased general permeability one day after the bleeding, which is the time at which our investigations were made, is evidently the temporary posthemorrhagic hydremia and anemia which bring about a certain degree of hypoxia; the early and complete restoration of the circulating blood volume and blood pressure, which is complete by this time, prevents any ischemia of the peripheral tissue.

SUMMARY

A study was made of the effect of various amounts of blood loss on general blood vessel permeability to substances of low molecular weight.

An increase in permeability was observed after 24 hours, the extent of the increase being proportional to the amount of blood lost.

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THE ANAPHYLACTIC REACTION AND TACHYPHYLAXIS TO EGGWHITE IN ALBINO RATS

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White rats are very sensitive to an initial parenteral injection of eggwhite [2, 5, 10, 15]. If 1 ml of the fresh substance is injected intraperitoneally into rats, an edema of the snout, tongue, paws, and clitoris develops. A hyperemia precedes the edema. The edema is reduced by antihistamine preparations [3, 6, 11]. Rats which have survived the anaphylactic reaction develop an insensitive condition, or one of reduced sensitivity to a second injection of considerably larger amounts of eggwhite [2, 15]. The rapid increases in resistance to various bacterial antigens is well known and has been called tachyphylaxis. However, very little work has been done either on the tachphylaxis of rats to eggwhite, or on the increased sensitivity of albino rats to a primary parenteral injection of this substance.

Recently many reports have been published on the part played by the hypophysis and adrenals in allergy and anaphylaxis [1, 4, 8, 9] However, many of these reports are contradictory, and the extent to which the hypophysical-adrenal system is involved is no means clear [7, 16].

The present work represents an attempt to determine the part played by the hypophysis and adrenals in the anaphylactic reaction and tachyphylaxis of albine rats to eggwhite.

METHOD

The experiments were carried out on more than 200 albino rats weighing from 80 to 150 g. Various doses of eggwhite were injected into the femoral vein to provoke the anaphylactic reaction. In acute experiments, records were made of the blood pressure in the carotid artery, and respiration. In order to obtain a more objective estimate of the degree of toxicity of eggwhite to rats, LD_{50} (medial lethal dose) was determined using the method of Reed and Muench [13]. Hypophysectomy was carried out using our modification of Smith's method [17] in which a paratracheal approach was made through the basioccipital bone. Indications that the hypophysis had been completely removed were that there was a twofold increase in the number of eosinophils, and an atrophy of the adrenals (on average 5.1 mg per 100 g weight in the operated animals as compared with 25.7 mg per 100 g weight in the controls). The adrenals were removed from the dorsal side by means of an incision along the ribs. The operations were carried out under ether anesthesia, and the estimation of LD_{50} made in the unanesthetized animals.

RESULTS

1. The anaphylactic reaction to eggwhite. Twenty-four acute experiments on albino rats were carried out. Intravenous injection of small amounts of eggwhite (0.2-1.5 ml per 100 g weight) produced the symptoms similar to those found in anaphylactic shock in sensitized guinea pigs. After 15-20 seconds, an increase in blood pressure of 10-56 mm mercury, and an increased respiration were observed; after 1-1/2 minutes the blood pressure gradually fell, sometimes to zero, but when the dose was not lethal, it recovered quite rapidly (Fig. 1).

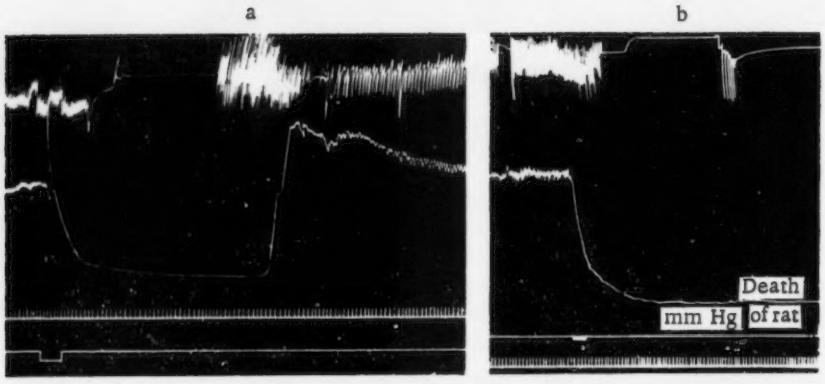

Fig. 1. Changes in the blood pressure and respiration in albino rats given eggwhite intravenously. a) intact animal; b) hypophysectomized animal. Curves, from above downwards: respiratory movements, blood pressure, time (2 seconds), stimulus marker — injection of 1 ml of eggwhite into femoral vein.

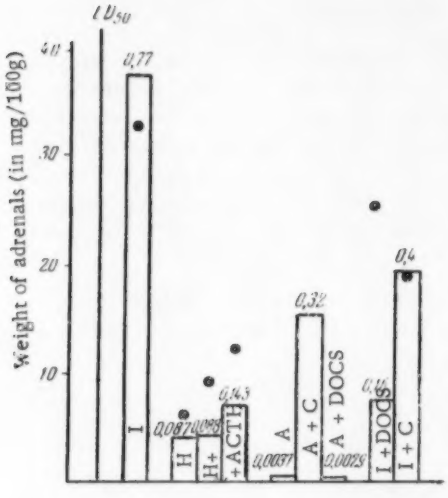

Fig. 2. Value of the LD_{50} for eggwhite and average weight of adrenals in rats in different experiments. Symbols: I) intact animals; H) hypophysectomized aninals; columns — value of LD_{50}; black dots — average weight of adrenals; ACTH — adrenocorticotropic hormone; A) adrenalectomy; C) cortisone; DOCS desoxycorticosterone.

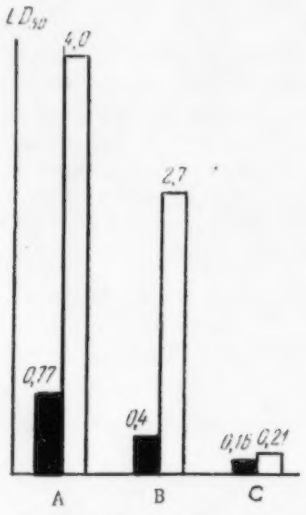

Fig. 3. Value of the LD_{50} in rats for the initial injection of eggwhite, and after tachyphylaxis had developed. Notation: a) intact animals; b) animals receiving cortisone; black columns refer to initial injection of eggwhite; white columns to tachyphylaxis; c) animals receiving desoxycorticosterone.

To determine the LD_{50}, 16 rats were divided into 4 groups of animals of approximately the same weight. Injections were made of eggwhite into the femoral vein, increasing in geometrical progression in the successive groups by a factor of 1.5, so that the amount given were 0.6, 0.9, 1.35, and 2 ml per 100 g weight. Two to three minutes after the injection, a hyperemia of the snout and paws developed, and this was followed by a cyanosis and edema. The hyperemia and edema were best shown in animals given small doses, and the cyanosis best in

those receiving the greatest amounts of eggwhite. After a few seconds, or later, the animals became breathless and restless. After 3-15 minutes, when the shock was severe, they fell on their side and died in convulsions. Quite frequently the first symptoms passed off, and death occurred 30 minutes or even several hours after the injection. Postmortem examination showed severe hyperemia and edema of the intestine. The LD_{50} for these animals was 0.77 ml per 100 g body weight (Fig. 2).

2. The hypophysis and the anaphylactic reaction. In 11 acute experiments, we observed that hypophysectomy increases the sensitivity of rats to eggwhite. After it had been injected intravenously, the blood pressure usually fell rapidly almost to zero, respiration stopped, and the reaction often ended in the death of the rats. Small doses of eggwhite were sufficient to cause death; injection of adrenocorticotropic hormone (ACTH) did not always prevent death (see Fig. 1, b).

To obtain further information on this problem, we determined the LD_{50} in a group of hypophysectomized animals, some of which were subsequently treated with ACTH, while the others received no further treatment. The experiment was performed on the 25-27th day after hypophysectomy. In the operated rats the LD_{50} was 8.8 times less than in healthy animals (0.087).

Different doses of ACTH given at different times to the operated animals did not restore the reduced resistance in the hypophysectomized rats. In those which received 4 units per 100 g body weight of ACTH 7 days before the experiment, the LD_{50} was the same as in the hypophysectomized group. The weight of the adrenals of this group was, on average, equal to 9.1 mg/100 g body weight. In another group of hypophysectomized animals given 1 unit per 100 g of ACTH for 21 days before the experiment, the LD_{50} showed some tendency to increase, although it did not regain the initial value. The weight of the adrenals in this group was also increased, on average to 12 mg per 100 g body weight.

3. The adrenals in the anaphylactic reaction. The adrenals play a far greater part than the hypophysis in the anaphylactic reaction in rats. Removal of the adrenals increases the sensitivity to parenteral injection of eggwhite 200 times; 6 days after the operation, the LD_{50} in these animals was 0.0037 ml per 100 g weight (see Fig. 2).

Injecting 2.5 mg per 100 g body weight of cortisone into the operated animals for 6 days caused a marked increase in resistance (LD_{50}=0.32 ml per 100 g body weight), although it did not return to the original level. A dose of 1 mg per 100 g of desoxycorticosterone (DOCS) for 6 days in the operated group caused a further reduction of the LD_{50} to eggwhite (LD_{50}=0.0029 ml per 100 g body weight; see Fig. 2).

In the next set of experiments, cortisone and DOCS were injected in the same amounts (1 mg per 100 g body weight) into the unoperated group for 7 days. Both substances reduced the rats' resistance to eggwhite; with cortisone the LD_{50} was 0.4 ml, with DOCS it was 0.16 ml per 100 g; see Fig. 2.

4. Tachyphylaxis to eggwhite. In acute experiments, we observed several times that animals which had survived the anaphylactic reaction became more resistant to the same protein subsequently. Tachyphylaxis develops almost immediately after the injection, and is maintained for several days.

In experiments specially designed to induce tachyphylaxis, 24 rats received injections of 0.6 ml per 100 g body weight, which is less than the LD_{50}. After 6 hours, the LD_{50} for the remaining 16 rats was determined. It was found to have a value of 4 ml per 100 g weight, i.e. it was more than five times greater than the original LD_{50} (Fig. 3, a).

The next step was to determine the effect of the adrenal hormones on tachyphylaxis to eggwhite. For this purpose, 2.5 mg per 100 g body weight of cortisone or 1 mg per 100 g weight of DOCS was injected for 7 days into intact rats. Tachyphylaxis was induced by injecting 0.2-0.4 ml of eggwhite. The LD_{50} for a second injection of eggwhite was then determined 6 hours after the first anaphylactic reaction. It was found that animals treated with adrenal cortical hormones developed a tachyphylaxis (Fig. 3, b), while in DOCS treated animals the ability to do so was greatly reduced (Fig. 3, c).

Long ago, Selye [16] showed the effects of hormones of the adrenal cortex and hypophysis on the local manifestation of the anaphylactic reaction in rats (edema, hyperemia). In the present study, besides confirming Selye's results, particular attention has been paid to the general reaction of the rats, which consisted of a change in blood pressure, respiration, and the appearance of tachyphylaxis. For the most part, our results agree with those of Selye: adrenalectomy markedly reduces, and cortisone treatment increases, the rats' resistance to eggwhite; desoxycorticosterone has no definite effect.

In our experiments, hypophysectomy increased the sensitivity to the first eggwhite injection, whereas according to Leger and Masson, there is no alteration in the degree of edema in the hypophysectomized animals. The disagreement is obviously due to the fact that these authors carried out the experiments on the ninth day after the operation, whereas ours were performed later, on the 25-27th day. Different degrees of atrophy of the adrenals can be observed at different times after the operation, and these play an essential part in the anaphylactic reaction of rats to eggwhite. It is important to note also that our method of determining the LD_{50} is more objective than is estimation of the development and intensity of edema. In our experiments, injections of ACTH did not restore the reduced resistance to eggwhite in the hypophysectomized rats. The explanation may be that the dose of hormone was insufficient, or that it was given too late after the operation to prevent atrophy of the adrenals following removal of the hypophysis. The weight of the adrenals in these animals remained 2-3 times below the normal value.

SUMMARY

Anaphylactic shock in rats may be provoked by an initial intravenous injection of fresh eggwhite. Previous administration of cortisone or desoxycorticosterone reduces the animals' resistance to the initial eggwhite injection, while adrenalectomy sharply decreases it.

Cortisone restores the resistance reduced by adrenalectomy, while DOCS produces no appreciable effect.

Resistance to eggwhite is also decreased on the 25th day after hypophysectomy, although to a smaller extent than in adrenalectomy.

Different doses of ACTH employed at various intervals had no visible compensatory effect.

The condition of tachyphylaxis occurred after the first injection of egg white. Preliminary cortisone and DOCS administration more or less preserved the ability to produce a tachyphylactic response.

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SOME OBSERVATIONS ON ALLERGY IN NEURITES

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There are comparatively few accounts of experimental studies of allergy in nervous conductors [2, 3, 4, 5, 10, 11, 12, 13], and very little is known about how this process develops.

We have studied changes in the functional condition of the neuromuscular apparatus produced by the direct action of an antigen on the nerve. The activity of the nerve could be inferred from tetanic muscular contraction produced by stimulating the nerve at various frequencies. This method of studying the activity of nervous structures is used both clinically and experimentally [1, 6, 8, 9].

METHOD

The experiments were carried out on normal and on sensitized guinea pigs. Normal horse serum was used for sensitization. An injection of 0.1 ml was given subcutaneously, by the method usually used to sensitize guinea pigs. The weight of most of the guinea pigs was 400-500 g. Guinea pigs which had been sensitized for from 7 to 51 days were taken. The lability of the neuromuscular apparatus was determined from the frequency of stimulation applied to the nerve. The index of the pessimal effect was the reduction of the tetanus of the muscle. In our experiment, we recorded the contractions of the tibialis anterior muscle while stimulating the peroneal nerve. Reduction of tetanus occurred at different frequencies; in the great majority of guinea pigs the value of this frequency was 50-80 impulses per second. The threshold stimulus for the nerve was found at a constant frequency of 25 impulses per second, and varied between 0.5 and 0.7 v. For electrical stimulation, a value 25% above threshold was used. The experiments were carried out on spinal animals in order to avoid any effect of the anesthetic on the lability of the nervous structures.

The spinal cord was cut between the cervical and thoracic cord. A preparation was made of the tibialis anterior muscle and the peroneal nerve. The peripheral end of the cut peroneal muscle was stimulated with a square-wave voltage at various frequencies. The contractions were recorded on a rotating drum kymograph. Ink-writing was used.

The experiment was carried out one hour after sectioning the cord. A series of 6-8 impulses at different frequencies was applied to the nerve. Stimulation lasted 3-5 seconds, and the intervals for 15-20 seconds. A rest period of 5-10 minutes then followed, after which a series of stimuli was once more applied. A wad soaked in antigen was placed on the nerve between the muscle and the electrode for 5 minutes. A test for sensitization was made by injecting the permissible dose of antigen [7]. In most cases, the effect of the antigen on the nerve of both limbs was studied in the same animal.

Nineteen experiments on 10 unsensitized animals and 45 on 17 sensitized guinea pigs were carried out.

RESULTS

In the unsensitized guinea pigs, in most cases, when the antigen was applied to the nerve, no definite changes in the neuromuscular apparatus occurred. The muscular contractions resulting from stimulating the nerve at diff-

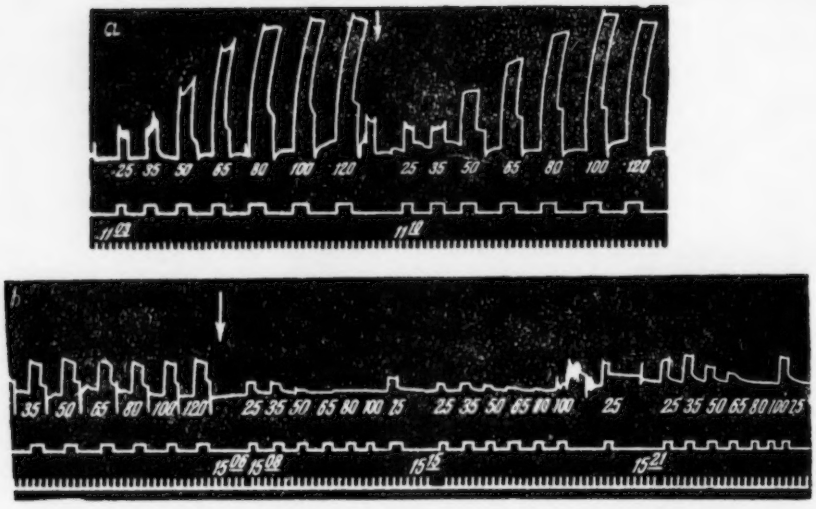

FIGURE. Muscular contractions on stimulating the nerve at different frequencies in (a) the unsensitized and (b) the sensitized guinea pigs. Curves, from above downwards: muscular contractions; nerve stimulation; figures indicate number of stimuli per second; time marker (3 seconds); ↓— moment of stimulation (a wad soaked in normal horse serum applied to the nerve).

erent frequencies were the same before and after applying the antigen (see Figure, a).

Applying the antigen to the nerve of the sensitized animals caused marked changes in its activity (see Figure,b). When the nerve was stimulated in the same way as before, the resulting muscular contractions were entirely different. They were reduced in height, and in some cases, immediately after the application of the antigen to the nerve, no contractions occurred, and appeared only after an interval of several minutes. This result indicates a suppression of nervous conductivity due to antigen action. If the nerve was stimulated below the region where the antigen had acted, muscular contractions were evoked. Thus, conductivity is disturbed only in that portion of the nerve to which the antigen had been applied.

When antigen is applied to a nerve, changes in the reponse depend also on the frequency of stimulation. At first there is a change to high frequency stimulation. This result indicates that the lability of the nerve is reduced, and falls below even the value for the lability of the neuromyal junction, because if the nerve is stimulated below the portion to which the antigen has been applied, that is,nearer to the muscle, the normal muscular contraction takes place.

The change in nervous conductivity was temporary, and disappeared when the nerve was washed with physiological saline. The described changes in the nerve could be obtained several times. In recovery, conductivity returned first for low frequencies, and later for high.

Thus, in nerve allergy, secondary changes in the activity of the neuromuscular apparatus, associated with a disturbance of the conduction of impulses from the central nervous system may occur.

We have shown previously [7] that when antigen is injected into the blood of a sensitized animal, it depresses the lability of the neuromuscular junctions.

The results obtained indicate that in the allergy of neurites and in the associated impairment of activity of the neuromuscular apparatus, in addition to other causes, the direct action of the antigen on the nerve fibers may also play a part in a sensitized animal.

SUMMARY

Experiments were performed on guinea pigs. It was shown that the antigen,normal horse serum,acted on the nerve of a sensitized animal to depress its conductivity. The conduction of high frequency impulses was the first

to be affected. No such changes occurred when the antigen acted on the nerve of a non-sensitized animal.

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THE PATHOGENESIS OF PULMONARY EDEMA CAUSED BY SILVER NITRATE

COMMUNICATION II. CIRCULATORY DISTRUBANCES AND PULMONARY EDEMA

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The problem of the significance of circulatory disturbances in pulmonary edema is an old one, but it still awaits solution. The edema is often associated with circulatory disturbances but this fact by itself does not explain the causal relationships.

It has been shown that for many clinical and experimental forms of pulmonary edema, the circulatory disturbances constitute the principal symptom [1, 4, 5, 6, 7, 10, 11, 12]. In other cases such disturbances develop only in the terminal stages of the illness, and appear to be secondary to the edema [2, 8, 9].

In the present study, to determine the relationships between the two conditions, we made use of pulmonary edema induced by silver nitrate.

After injecting this substance, in addition to the edema, severe circulatory disorders develop [3].

METHOD

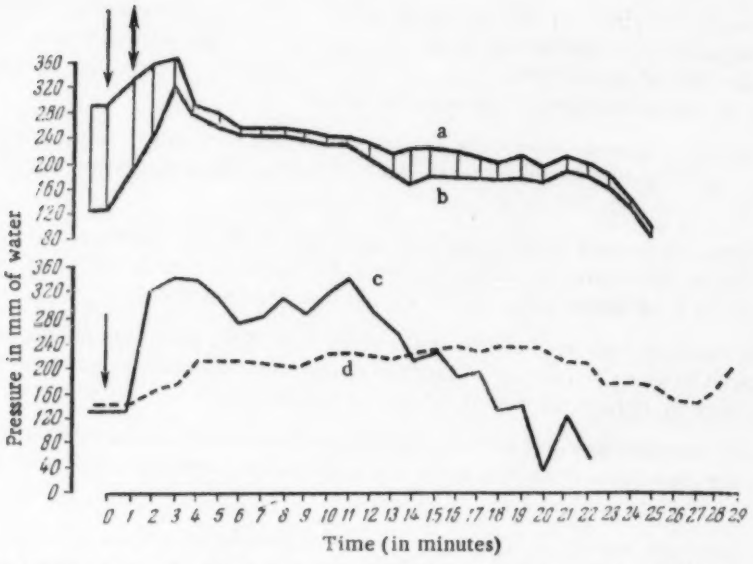
Ten adult dogs were used in the experiments. As a control, 27 animals were used, their treatment being as described in the Tables under the headings "Control No. 1" and "Control No. 2".

One group of 5 animals received the silver nitrate by inhaling it in the form of an aerosol. For this purpose a glass atomizer was filled with a 4% solution of silver nitrate. A bent glass cannula was connected to a diaphragm pump, and inseted into a slit in the trachea, the diameter, length, and angle at the bend chosen so that for the particular pumping rate and atomizer aperture, an appropriate atomizing speed and particle diameter were obtained.

To eliminate any possible effect due to osmosis, in one experiment. 4% Na Cl solution was substituted for the 4% $AgNO_3$.

In another group of 5 dogs, 3.2 mg/kg of 0.4% silver nitrate solution was injected intravenously.

To maintain the pressure in the pulmonary artery near to its original level, the chlorvinyl catheter into the pulmonary artery to measure the pressure was also connected to a vertical glass tube whose height could be adjected. The whole system was filled with physiological saline. The side opening in the tube was placed at a level such that blood entering the tube from the catheter could not escape through it. As soon as the pressure in the pulmonary artery was raised after injecting the silver nitrate, the system was closed, and blood from the side hole in the tube flowed out into a special receiving funnel, and thence into the venous system of the greater circulation.



Pressure in the pulmonary artery under different experimental conditions (average figures). Curves, from above downwards: a) systolic and b) diastolic pressure in the pulmonary artery in an experiment in which the increase in pressure is eliminated artificially; c) mean systolic and diastolic pressure in the pulmonary artery following intravenous injection of silver nitrate only; d) mean systolic and diastolic pressure in the pulmonary artery when silver nitrate is inhaled. The abscissa shows average survival time of the animals in minutes after injecting silver nitrate (moment of injection shown by arrow ↓), the double arrow indicates the moment at which the mechanism for preventing blood pressure increase is put into action.

Ratio of Weight of Body, Heart, and Dried Lung Residue to Fresh Lung Weight

Series	Number of expts.	Ratio of lung wt. (in g) to body wt. (M ± m)	Ratio of lung wt. (in g) to heart wt. (M ± m)	Dried lung residue as percentage of fresh weight
Silver nitrate inhaled	5	23.00 ± 2.82	2.70 ± 0.31	13.40 ± 0.84
Blood pressure in pulmonary artery maintained at approximately original level	5	25.30 ± 2.30	3.12 ± 0.37	15.35 ± 0.79
Control No. 1				
Silver nitrate injected intravenously, no other operation	20	38.55 ± 1.60	4.68 ± 0.25	13.40 ± 0.35
Control No. 2				
Intact animals, killed by electrification	7	9.35 ± 0.83	0.96 ± 0.05	20.10 ± 0.41

Heparin was added, and the surface of the apparatus in contact with the blood was covered with silicone.

RESULTS

The table and figure show changes in the relative weights of the lungs and in the pressure of the pulmonary artery. From the figure (d), it can be seen that for pulmonary edema to develop, it is not necessary for the pressure in the pulmonary artery to be increased.

In the inhalation experiment, in spite of the fact that there was no significant increase in the pressure in this artery, the degree of edema as measured in terms of the dry lung tissue was the same as that in Control No. 1 (see Table). The smaller ratio of lung weight to body and heart weight than in Control No. 1 indicates that the lungs of the animals in this series contained considerably less blood.

In the experiments where increase in pulmonary artery pressure was eliminated, edema developed just the same. This can be seen by comparing the results for the lungs of the animals in this series, with those of normal animals (Control No. 2).

In these experiments, the pressure in the pulmonary artery was less than the initial systolic pressure, except for the small initial increase, which may be partly ascribed to the failure to operate the pressure levelling mechaism at precisely the amount at which the silver nitrate was injected (see figure).

The ratio of lung weight to body and heart weight, and the ratio of the dried lung residue to the raw weight, as found in the experiment in which an isosmotic solution of sodium chloride was injected, showed that the values obtained do not differ appreciably from the normal values (Control No. 2).

Both we and A. M. Kotovshchikov [3] have shown that pulmonary edema induced by silver nitrate may occur without any increase in pulmonary artery pressure and without any appreciable circulatory disorders developing.

We have shown previously that after a silver nitrate injection there is no increase in the pressure in the pulmonary vein, but that on the contrary, it was reduced. Since this reduction in pressure could not be a pathogenic factor in pulmonary edema, it has not been recorded in the present experiments.

Neither we nor A. M. Kotovshchikov [3] were able to reproduced the previously mentioned experiments in which there were no circulatory disturbances in the pulmonary circulation For this purpose, certain special conditions are required which it is not possible to determine precisely.

It must be supposed that one such condition is a partial failure of cardiac muscle. It may be associated with severe operational trauma, or with the effect of the anesthetic, or with any other factor which will interfere with the action of the heart before the injection of the silver nitrate.

When giving the silver nitrate by inhalation, it was not possible to induce pulmonary edema without any disturbance to the lesser circulation, but the increase in pressure in the pulmonary artery when using this method was very much less than when the silver nitrate was injected intravenously(see figure).

The extent of the pulmonary edema as determined from the dry pulmonary tissue residue did not differ from that produced by intravenous injection. The smaller ratio of lung weight to body and heart weight in the experiments in which the preparation was inhaled, is evidently not due so much to the accumulation of transudate, as to the different degree of engorgement of the lungs with blood.

In the other set of experiments, in which pressure changes in the pulmonary artery were artificially excluded, it was shown that the development of the condition does not depend upon this pressure increase. The slightly smaller degree of edema in this case is understandable, because with this method, inevitably a smaller amount of blood passes through the lungs in unit time. It has long been known that a reduction in blood flow to the lungs during the development of edema will reduce the amount of transudate.

Probably the increased pressure in the pulmonary artery does not represent the principal factor causing the edema. We have shown previously that the observed circulatory disturbances cannot be caused by the edema of the lungs.

The edema and the circulatory disturbances are two separate consequences of the silver nitrate injection. Both processes also react upon each other, as can be seen from the results which have been presented.

SUMMARY

The effect of circulatory disturbances on pulmonary edema caused by silver nitrate injections in dogs was studied. Silver nitrate was inhaled or injected, and arrangements were made for artificially preventing an increase in pressure in the pulmonary artery when required; it was shown that circulatory disturbance is not an important factor in pulmonary edema.

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RESTORATION OF STORED GLYCOGEN IN THE LIVER OF WHITE MICE FOLLOWING MUSCULAR STRESS WITH LIGATED PANCREATODUODENAL VEIN

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It has been shown in E. N. Speranskaya's laboratory that insulin reduces liver function. After ligature of the pancreatoduodenal vein in dogs and rabbits, there was a change in the blood sugar curves during muscular work [2]; when the orifice of this vein was transferred from the portal to the inferior vena cava in dogs, a change in the rate of synthesis of new ethereal sulfate compounds was observed [3, 4]. In white mice, after ligature of the pancreatoduodenal vein, there was a reduction both in the glycogen content and in the cholinesterase activity of the liver [1]. The liver glycogen fell by 40% between the 4th and 16th days after the operation.

We required to find how glycogen loss caused by muscular work in animals with impaired liver function is made good. For this purpose, the following investigation was undertaken.

METHOD

Four sets of experiments were carried out on 127 white mice, and the glycogen liver reserves were determined under various experimental conditions. In the first group the liver glycogen content was determined in normal mice after muscular work, in the second group it was measured after ligature of the pancreatoduodenal vein, and in the third group the liver glycogen content in mice kept on a normal diet and with a ligature of the pancreatoduodenal vein was found; in the fourth group the same quantity was measured in mice with the ligature during muscular work after a 2-hour fast.

Ligature of the pancreatoduodenal vein was made under ether anesthesia. As a rule, the animals tolerated the operation well. In the II, III, and IV groups, animals were chosen in which the ligature had been applied 8 days previously. It is at this period that the most marked liver glycogen changes occur. A mock operation was carried out on the control animals. Both control and experimental animals were kept under the same feeding and living conditions. Before the experiment, they were starved for 2 hours; they were then killed by a swift decapitation, the liver was removed, dried on filter paper, and two portions weighed out on a torsion balance. Later these were treated with boiling alkali. Glycogen was determined by Simonovich's method [8], as modified by A. M. Genkin [5].

The results were analyzed statistically, because the variations in liver glycogen in mice are considerable. The figures treated included glycogen values varying from 0.3-3 g%; Experiment IV was an exception, and here very small amounts of glycogen were studied.

According to Stolzmann, Blawaska and Roth [10], the average liver glycogen content for white mice is 1.5-3.5 g%; the range of variation is very much greater, being from 0.3 to 3.5 g%. Muscular stress was caused by the animals swimming in water at 28° for 1 minute with a 2 g load attached to the tail.

Liver Glycogen Content in White Mice under Different Experimental Conditions

Number, in sequence	Group I: Date of experiments	Group I: liver glycogen (in g%) experiment mice	Group I: liver glycogen (in g%) control mice	Group II: Date of experiments	Group II: liver glycogen (in g%) experiment mice	Group II: liver glycogen (in g%) control mice	Group III: Date of experiments	Group III: liver glycogen (in g%) experimental mice	Group III: liver glycogen (in g%) control mice	Group IV: Date of experiment	Group IV: liver glycogen (in g%) experimental mice	Group IV: liver glycogen (in g%) control mice
1	2/10/1958	0,49	1,71	11/11/1957	1,83	1,28	2/21/1958	0,42	1,93	4/1/1958	0,24	0,28
2	2/10/1958	1,01	1,85	11/11/1957	1,84	2,46	2/21/1958	0,63	2,87	" " "	0,26	0,34
3	2/10/1958	0,92	2,21	11/11/1957	1,20	2,68	" " "	0,61	1,97	" " "	0,21	0,37
4	2/12/1958	1,64	0,53	11/14/ "	0,66	1,88	" " "	0,32	1,95	" " "	0,22	0,87
5	2/13/1958	2,39	0,51	11/14/ "	0,49	1,88	" " "	1,11	1,77	" " "	0,28	0,39
6	2/13/1958	1,50	2,38	11/14/ "	0,91	1,38	" " "	0,67	1,14	" " "	0,25	1,85
7	2/14/1958	1,73	1,95	11/14/ "	0,76	1,22	3/24/1958	1,02	1,85	4/4/1958	0,14	1,85
8	2/14/1958	1,30	2,47	" " "	1,74	2,44	" " "	0,72	1,28	4/4/1958	0,14	0,51
9	2/14/1958	1,35	1,87	3/18/ "	0,50	1,98	3/28/1958	0,87	1,36	" " "	0,82	0,61
10	2/16/1958	0,90	1,29	3/18/ "	0,77	1,23	" " "	0,88	0,89	" " "	0,26	
11	2/27/1958	2,51	0,60	" " "	1,57	1,45	" " "	1,92	2,58	" " "	0,1	Average 0,73 g% ±0,15
12	2/27/1958	1,00	0,59	" " "	2,20	1,57	" " "	1,86	2,65	" " "	0,1	
13	2/27/1958	0,75	1,10	" " "	0,52	1,22	" " "				0,16	
14	2/27/1958	0,60	0,51	" " "	1,44	1,85						
15	2/28/1958	0,80	1,90	" " "	0,32			Average 0,86 g% ±0,26	Average 1,85 g% ±0,10		Average 0,24 g% ±0,03	
16	2/28/1958	0,44	2,57	" " "	0,50	Average 1,85 g% ± 0,30						
17	2/28/1958	0,38	0,72	" " "	1,93							
18	2/28/1958	0,44	0,64	" " "	1,62							
19	2/28/1958	2,50	0,54									
20	3/2/ 1958	1,48	1,19		Average 1,15 g% ± 0,24							
21	3/3/ 1958	1,95	1,11									
22	3/3/ 1958	0,33	1,06									
23	3/3/ 1958	0,40	1,68									
24	3/3/ 1958	0,76										
25	3/11/1958	0,98	Average 1,31 g% ± 0,20									
26	3/11/1958	0,37										
		Average 1,11 g% ± 0,10										

RESULTS

In Group 1, 49 experiments were carried out, 26 with muscular work, and 23 without (controls). The results showed that the glycogen content of the control group was 1.31 g%, and that of the experimental group 1.11 g% (see Table). Thus the moderate amount of muscular work entailed by swimming for one minute with a 2g load causes a 15% fall in liver glycogen. Their results agree with published figures [7, 10 and others].

In group II, 32 experiments were performed, 18 on mice with a ligature of the pancreatoduodenal vein, and 14 on controls. The results are given in the table. It can be seen that the average liver glycogen value in the control mice was 1.85 g%, and in the experimental group 1.15 g%, i.e. the operation reduced liver glycogen by 0.7 g% or 38%; this agrees completely with results which we obtained previously [1]. Thus, breaking the path by which insulin reaches the liver causes a greater reduction in glycogen (38%) than does moderate muscular exercise (15%).

In Group III, 24 experiments were carried out, and of these 12 were performed on animals with a ligatured pancreatoduodenal vein, and 12 on controls. Both groups were exercised by swimming. The animals were then kept for 2 hours under normal conditions and with normal feeding. It was found that after swimming, the liver of the experimental group contained 0.86 g% of glycogen, while the value for the control group was 1.85 g%.

In comparing results of the experiments in Group II and Group III, it can be seen that muscular work in animals with reduced liver function causes a glycogen deficit which is not made good in 2 hours, while in the control group a 2-hour normal feeding period is sufficient for a complete restoration of the original glycogen level in the liver.

In Group IV, 22 animals were used, and of these 13 were used for the experiment and 9 served as controls. As previously, the animals swam while loaded, after which they were starved for 2 hours until they were killed and the liver samples taken.

It is known that fasting causes a considerable liver glycogen loss [6, 9 and others]. As the experiments on Group IV showed, in the operated group, after muscular work and a 2-hour fast, the glycogen reduction was considerable, while in the control group it was less. After exercise, the average amount of glycogen in the liver of the operated animals was 0.24 g%, and in the controls 9.73 g%. It may be concluded that alteration of the usual route along which insulin passes to the liver not only reduces the amount of glycogen stored in the liver but also interferes with the building up of further supplies, thus indicating an impaired hepatic function.

SUMMARY

Alterations were made to the route by which insulin entered the liver, and stored glycogen investigated.

Ligature of the pancreatoduodenal vein caused a reduction of 38% in the amount of glycogen stored.

It was shown that moderate muscular exercise in operated animals causes a glycogen expenditure which is not fully compensated by the normal diet.

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BIOCHEMISTRY AND BIOPHYSICS

THE BIOLOGICAL IMPORTANCE OF VITAMIN P

COMMUNICATION I. DIURESIS AND SERUM PROTEIN COMPOSITION DURING ADMINISTRATION OF VITAMIN P

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(Received April 10, 1959. Presented by Active Member AMN SSSR S. E. Severin)

There are only fragmentary reports in the literature on the mechanism of the physiological action of compounds exhibiting vitamin P activity.

Ya. N. Khodzhai [1] studied the diuretic action of flavones, isolated from the everlasting flower, in experiments on rats. He found that the volume of diuresis depended on the dose of flavone. When given internally in a dose of 3-6 mg/kg body weight, flavone caused an increase in the excretion of urine per hour of 12-13%, but did not alter the total urinary output during the period of observation. Doses of 7.5-10 mg/kg caused an increase in the volume of urine excreted during the first hour of 130-150% and also an increase in the total urinary output during the period of observation.

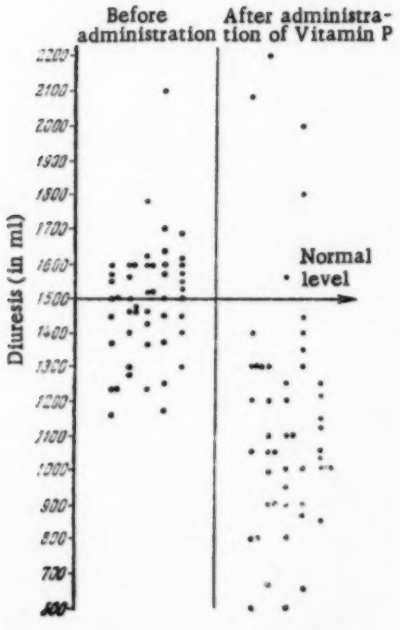

Fig. 1. The effect of vitamin P on diuresis in man.

These findings suggested that it might be interesting to test the influence of vitamin P on diuresis in man. As a source of vitamin P we used a green tea preparation, produced by the Moscow Chemopharmaceutical Factory, containing 98.6% of tea catechols.

The 1.4% of impurities evidently consisted of mineral substances, chlorophyll and traces of caffeine, possessing an antidiuretic action.

When the vitamin P preparation was used in the treatment of Far Eastern hemorrhagic fever (patients received 150 mg of vitamin P twice a day for 2-5 days), we observed a fall of 28-31% in the diuresis and an increase in the toxicosis. Since the results obtained disagreed with Ya. N. Khodzhai's findings mentioned above we decided to test the influence of our vitamin P preparation on diuresis in healthy human subjects. Observations were carried out on 50 healthy adult males (students), aged from 20 to 3 years. All the persons investigated received 300 mg of vitamin P (150 mg twice a day).

TABLE 1

The Effect of Repeated Administration of Vitamin P on Diuresis

Serial no.	Subject	Diuresis (in ml)					
		before administration of the vitamin	day after administration of the vitamin				
			1st	2nd	3rd	4th	5th
1	F	1 635	1 420	1 265	1 130	1 010	860
2	K	1 690	1 500	1 485	1 240	1 085	950
3	S	1 475	1 280	1 300	1 120	1 020	980

TABLE 2. The Effect of Vitamin P Saturation on the Concentration of Serum Protein and its Fractions in the Blood of Rabbits

Serum proteins	Time of investigation	Serum protein concentration (in g %)		Concentration of protein fractions (relative %)	
		control	experiment	control	experiment
Total protein	I	6,53±0,58	7,30±0,30	—	—
	II	7,23±0,32	6,64±0,46	—	—
Albumins	I	3,90±0,17	4,20±0,20	59,70±2,60	57,50±2,74
	II	4,23±0,12	3,95±0,15	58,50±1,66	59,48±2,25
α_1-globulins	I	0,45±0,08	0,50±0,06	7,89±1,38	6,85±0,96
	II	0,66±0,06	0,40±0,07	9,12±0,83	6,02±1,05
α_2-globulins	I	0,64±0,12	0,62±0,09	8,80±1,64	8,50±1,23
	II	0,63±0,12	0,41±0,10	8,71±1,65	6,17±1,50
β-globulins	I	0,68±0,19	0,89±0,12	10,40±2,90	12,22±1,64
	II	0,67±0,05	0,94±0,08	9,26±0,70	14,18±1,21
γ-globulins	I	0,86±0,02	1,09±0,05	13,21±0,31	14,93±0,69
	II	1,01±0,10	0,94±0,04	14,41±1,39	14,15±0,60

Note. I) before administration, II) after administration. Vitamin P was given to the experimental animals, physiological saline to the controls.

The diuresis was measured during the 24 hours preceding the administration of vitamin P and during the 24 hours afterwards. The subjects were kept in identical conditions (attending their classes), on an ordinary diet, and with a steady water intake. The results of the investigations are shown in Fig. 1.

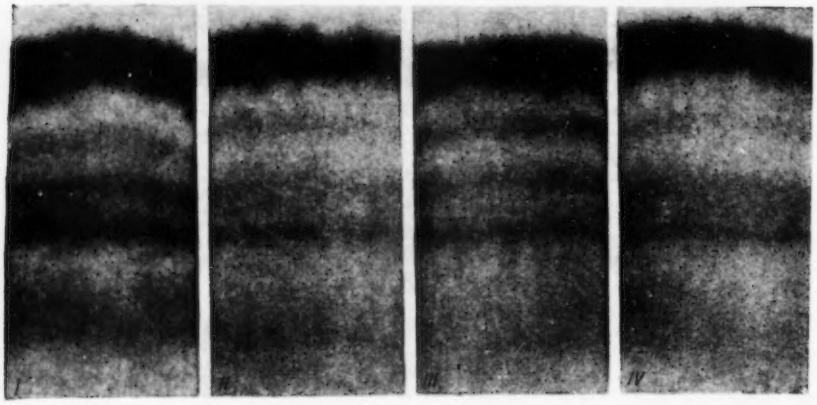

Fig. 2. Electrophoregram of the serum of the rabbits. I and III – before taking vitamin P; II and IV – after taking vitamin P.

Administration of vitamin P was found to lower the mean daily urine output from 1434 to 1145 ml, i.e., by an average of 21%. After the repeated administration of vitamin P (saturation was achieved after giving a daily dose of 300 mg of vitamin P for 5 days) a fall in diuresis developed, and as shown by the figures in Table 1, this was more sharply expressed.

The subjects observed a diminshed sense of thirst.

Besides investigating the effect of vitamin P on diuresis, we also studied its effect on the protein fractions of the blood in rabbits. For a period of 5 days 10 rabbits were given by mouth 2.5 ml of a solution of vitamin P, equivalent to 25 mg/kg body weight/day. Samples of 1 ml of blood were taken from the rabbits before receiving vitamin P and after saturation with the vitamin, from the auricular vein, and the total serum protein was estimated refractometrically, and its fractions determined by the method of paper electrophoresis (Fig. 2).

In 5 other rabbits (controls) the composition of the serum proteins was also investigated. These rabbits were given physiological saline by mouth, in a daily dose of 2.5 ml, after which the serum of these rabbits was again investigated.

The results of these investigations are shown in Table 2.

It can be seen from Table 2 that the concentration of total protein and of α_2- and γ-globulins falls after administration of vitamin P for 5 days, whereas the concentration of β-globulins rises slightly, although these changes were so slight that it is difficult to ascribe any importance to them. The effect of vitamin P on diuresis in man is so marked that it must be taken seriously into consideration when this vitamin is prescribed for patients.

SUMMARY

It has been demonstrated that vitamin P possesses a pronounced antiduretic effect in man.

When the vitamin P was administered to rabbits the following changes were noted in their blood serum: a slight decrease in the concentration of the total protein, α and γ-globulin fractions and some increase in the β-globulins.

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THE EFFECT OF VITAMIN P ON THE PERMEABILITY OF THE VESSELS OF THE ANTERIOR CHAMBER OF THE EYE IN RABBITS

(PRELIMINARY COMMUNICATION)

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(Received November 22, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

This investigation was to study the changes taking place in the permeability of the vessels of the anterior chamber of the eye to fluorescein, in rabbits receiving a daily dose of 500 mg of vitamin P (tea catechols) by mouth.

EXPERIMENTAL METHOD

The permeability was measured fluorometrically by E. G. Mikheeva's method. The fluorescein solutions used were in dilutions of 1:48 000 to 1:5 120 000.

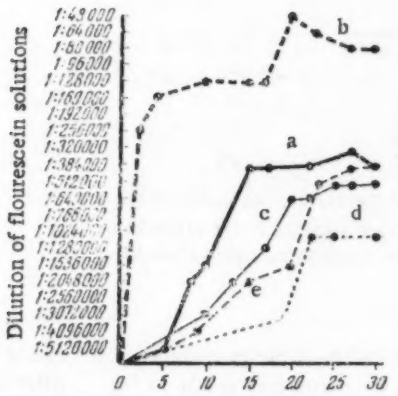

Fig. 1. Changes in the permeability of the vessels of the anterior chamber of the eye in the experimental rabbit no. 712. Conventional signs: a) in the control rabbit; b) after subconjunctival injection of ethylmorphine hydrochloride; c) 3 days after administration of 500 mg of vitamin P; d) 6 days after administration of 500 mg of vitamin P. Time of investigation of fluorescence 30 minutes.

Fluorescein was injected intravenously into rabbits in doses of 10 mg/kg body weight, i.e. 0.2 ml of 5% solution per kg body weight. The fluorescence of the humor in the anterior chamber of the eye was measured every 2 minutes during the 30 minutes after injection of the dye into the animal. The biological action of vitamin P can be shown both experimentally and clinically against a background of increased permeability of the vascular wall. The increased permeability of the vessels, caused by injection of 1 ml of 3% ethylmorphine hydrochloride beneath the conjunctiva of each eye 15 minutes before the investigations, was therefore measured in each rabbit 48 hours after investigation of the normal permeability. The rabbits were used in the experiment only after the measurement of the normal and increased (after injection of ethylmorphine hydrochloride) permeability, and during this time they received vitamin P by mouth every day. Subsequent measurement of the permeability in the experimental animals were also carried out in the presence of increased permeability.

The experiments were performed on 11 chinchilla rabbits weighing from 2.5 to 3.0 kg. The experimental animals were under observation for from 10 to 25 days.

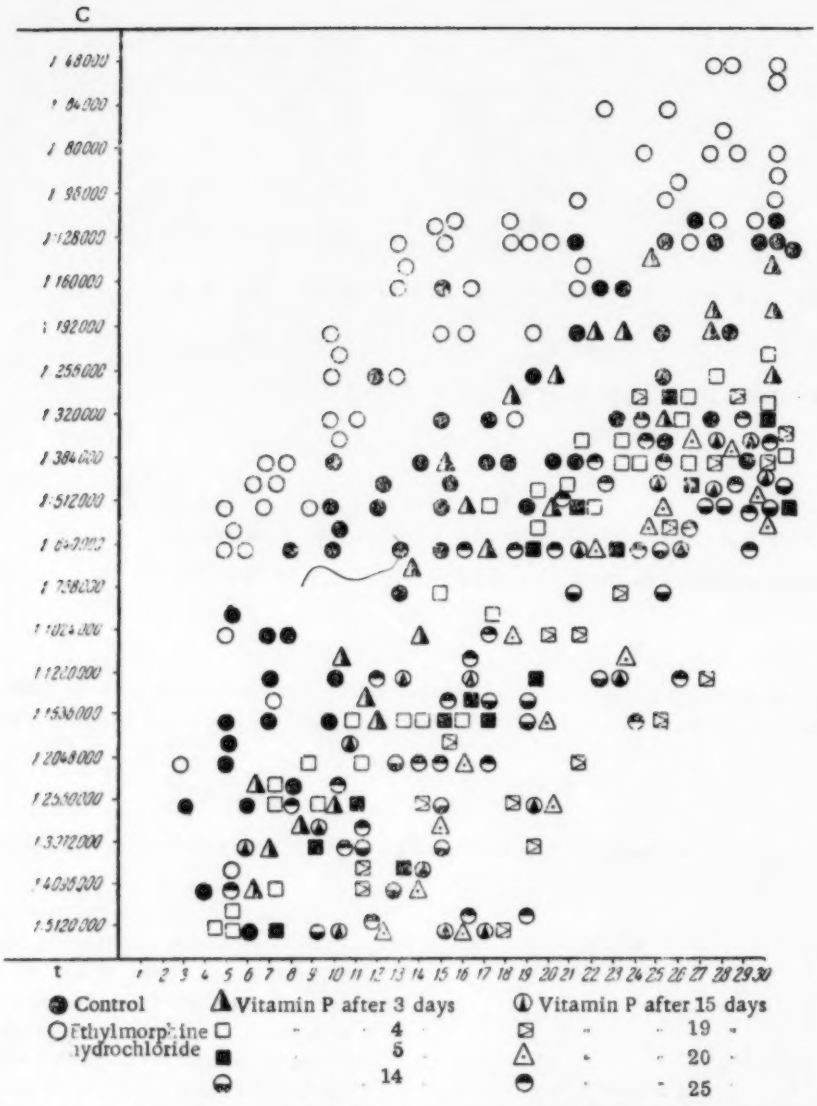

Fig. 2. Changes in the permeability of the vessels of the anterior chamber of the eye to fluorescein in 11 rabbits, in experiments in which vitamin P was given in a dose of 0.5 g after the subconjunctival injection of ethylmorphine hydrochloride.

EXPERIMENTAL RESULTS

Typical changes in the accumulation of fluorescein in the humor of the anterior chamber of the eye in rabbit No. 712, which received vitamin P daily in a dose of 500 mg, are shown graphically in Fig. 1. After only 3 days, the permeability of fluorescein in this rabbit was subnormal, and it remained at this level until the end of the experiment (6-10 days).

In the presence of increased permeability due to ethylmorphine hydrochloride the concentration of fluorescein in the humor of the anterior chamber was not less than 1 : 192 000 in the majority of experimental animals. The limit of accumulation of the dye in the humor of the anterior chamber under normal conditions in the experimental animals did not, as a rule, exceed a concentration of 1 : 256 000. The permeability of the vessels of the experimental animals receiving large doses of vitamin P to fluorescein was subnormal after 3 days, and remained at this level until the end of the experiment (Fig. 2).

The restoration of the normal permeability of the vessels of the anterior chamber of the eye to fluorescein depended on the dose of vitamin P. After a dose of 5 and 15 mg the permeability returned to normal in the majority of cases after 2 days. In some rabbits normal permeability was restored more slowly (after a dose of 5 mg – after 3 days, and after a dose of 15 mg – after 4 days).

On increasing the dose of vitamin P to 25 mg, the permeability of the vessels returned to normal in the majority of animals not before 5 days, and in one case 7 days from the time of administration.

The results obtained show that the biological effect of different doses of vitamin P is evidently due to the fact that its influence on the permeability of the vessels is closely connected with the dose given.

SUMMARY

Experiments were performed on 11 rabbits. The author studied the biological effect, of vitamin P on blood vessel permeability to fluorescein in the anterior eye chamber by comparative fluorometry. The accumulation of this dye in the aqueous humor was also studied in normal conditions and against the background of increased permeability caused by ethylmorphine hydrochloride.

Permeability of the vessels was found to be below normal 3 days after the administration of vitamin P (500 mg) and remained unchanged until the end of this experiment (25 days).

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PHARMACOLOGY

THE EFFECT OF ANALGESICS ON REFLEXES FROM THE PERICARDIUM AND THE HILAR REGION OF THE LUNGS DURING HYPOTHERMIA

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The use of analgesic drugs affecting the interoceptive reflexes in thoracic surgery has created interest in the study of the effect of these drugs during hypothermia. In the present work we investigated the action of morphine, promedol and phenadone on the reflex changes in the blood pressure in response to stimulation of the pericardium and the hilar region of the lung during hypothermia (30-32° and 25-27°).

EXPERIMENTAL METHOD

Experimental were carried out on 52 cats weighing from 2.5 to 4 kg. In order to produce hypothermia, the animals were anesthetized with urethane (1.2-1.4 g/kg) and placed in a double-walled metal bath, through which cold water was passed so as to maintain the body temperature of the animal at a constant level throughout the experiment.

Increase in the Depressor Vascular Reactions in Response to Stimulation of the Pericardium and Hilar Region of the Lungs under the Influence of Analgesics (as % of the Normal Value) at Different Body Temperatures (Mean Values)

Drug	Dose (in mg/kg)	36–38°		30–32°		25–27°	
		pericardium	hilum of the lungs	pericardium	hilum of the lungs	pericardium	hilum of the lungs
Morphine	2,0	150	183	155	124	138	139
	5,0	140	196	131	158	120	144
Promedol	1,0	126	138	114	134	100	100
	3,5	117	120	112	108	105	100
Phenadone	1,0	123	116	125	115	119	103

*Note. The value of the depressor reflex before injection of the analgesic (normal) is taken as 100%.

The pericardium was stimulated by inflating it with air through a special cannula. The degree of distension, which was measured by a water manometer, was constant in all the experiments (60-80 mm of water).

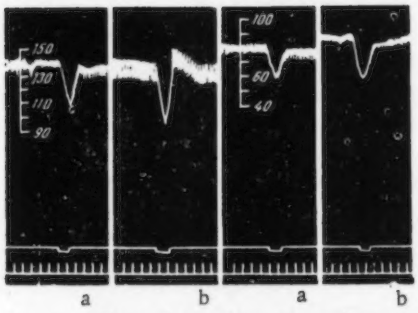

Fig. 1. Effect of morphine (2 mg/kg) on the reflex changes in the blood pressure in response to stimulation of the pericardium at a normal temperature (left half of kymogram) and during hypothermia to 25-27° (right half of kymogram). a) before injection of the drug; b) 60 minutes after injection. Significance of the curves (from above down): blood pressure, stimulus marker, time marker (5 seconds).

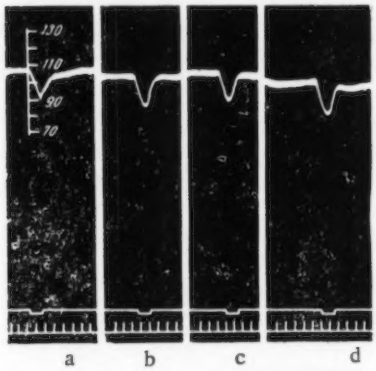

Fig. 2. Effect of promedol (1 mg/kg) on the reflex changes in the blood pressure in response to stimulation of the hilar region of the lungs during hypothermia to 25-27° a) before injection of promedol; b) 10 minutes after injection; c) 30 minutes after; d) $1^1/_2$ hours after. Significance of the curves as in Fig. 1.

cooled to 30-32° (see table).

The hilar region of the right lung was stimulated by being stretched with a weight of 115 g through a system of pulleys. The blood pressure was recorded in the usual manner. The analgesics were injected intravenously in the following doses: morphine 2 and 5 mg/kg, promedol 1 and 3.5 mg/kg and phenadone 1 mg/kg.

So that the results obtained should be comparable, we first investigated the effect of hypothermia on the reflex lowering of the blood pressure in response to stimulation of the receptive zones of the pleural cavity, and the effect of analgesics on this reaction at a normal temperature–36-38°. The action of the analgesics was then studied during hypothermia (30-32° and 25-27°). In order to compare the results obtained, the value of the depressor reaction before and after injection of the analgesic was calculated as a percentage of the initial (at the moment of stimulation) level of the blood pressure. The ratio between the value of the vascular reaction (in %) after injection of the analgesic and the value of the vascular reaction (in %) before injection of the drug was used as an index of its effect on the depressor reflex.

EXPERIMENTAL RESULTS

In all the experiments on animals at normal temperature and during hypothermia to 30-32°, the magnitude and character of the reflex response to stimulation of the receptor zones were almost identical. During hypothermia to 25-27° a fall in the reflex responses to two-thirds their previous magnitude was observed. An increase in the intensity of stimulation led to an increase in the depressor reaction. Restoration of the initial level of the reflexes usually took place after warming to 34-35°.

The effect of the analgesics investigated on the reflex reactions in response to mechanical stimulation of the pericardium and of the hilar region of the lungs followed the same pattern, and was expressed as an increase in the depressor reflex.

Injection of morphine in doses of 2 and 5 mg/kg, at 36-38°, caused an increase in the depressor reaction after 10-15 minutes to 125-136% in response to stimulation of the pericardium, and to 160-194% in response to stimulation of the hilar region of the lungs (the magnitude of the depressor reaction before injection of the analgesic was taken as 100%). Maximum increase in the depressor reaction was observed 50-60 minutes after injection of morphine (see table) and it continued for $1^1/_2$-2 hours (dose 2 mg/kg) or much longer (dose 5 mg/kg). A similar effect was shown by morphine in response to stimulation of the paricardium in animals

During hypothermia to 25-27° the action of morphine in enhancing the vascular reflexes was appreciably weakened. This is illustrated by Fig 1, in which it is shown that the action of morphine in a dose of 2 mg/kg at a normal temperature was more pronounced than during hypothermia to 25-27°.

Hypothermia to 30-32° and 25-27° appreciably lowered the effect of morphine (2 and 5 mg/kg) on the reflexes from the hilar region of the lungs. Whereas, for instance, in a dose of 5 mg/kg and at a normal body temperature, morphine almost doubled the magnitude of the depressor reaction, during hypothermia its action was less pronounced (see table).

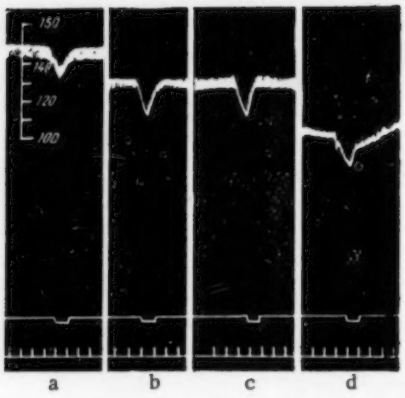

Fig. 3. Effect of phenadone (1 mg/kg) on the reflex changes in the blood pressure in response to stimulation of the hilar region of the lungs during hypothermia to 30-32°. a) before injection of the drug; b) 10 minutes after injection; c) 60 minutes after; d) 2 hours after. Significance of the curves as in Fig. 1.

The effect of promedol on the reflex changes in the blood pressure in experiments on animals at the normal temperature and in hypothermia differed from that of morphine under the same conditions. In the first place, the action of promedol in doses of 1 and 3.5 mg/kg in enhancing the depressor vascular reflexes was almost identical whatever the range of temperatures. Secondly, in the doses which we studied, promedol had a weaker effect on the reflex changes in the blood pressure than did morphine at the same temperatures. Thirdly, in contrast to morphine, the action of promedol was diminished in parallel with the fall in body temperature, and in hypothermia at 25-27°, generally speaking, it was absent (Fig. 2).

The effect of phenadone (1 mg/kg) on the vascular reflexes in response to stimulation of the two receptor zones at the normal body temperature and in hypothermia differed from the action of the two previous analgesics. At all body temperatures studied, phenadone had an equally feeble effect on the depressor reactions (see table and Fig. 3). Its action was brief duration, and usually passed off from 1-$1\frac{1}{2}$ hours after administration.

It must be pointed out that our experiments at different temperatures failed to show any relationship between the degree of enhancement of the depressor reaction and the dose of analgesic. On the contrary, in large doses, morphine and promedol had a less marked enhancing action on the vascular reflexes than in doses of 1-2 mg/kg. In the experiments with phenadone a similar picture was seen. This was shown by the results of additional experiments during hypothermia to 32-33°, in which no sharp increase in the enhancing effect was observed when phenadone was given in repeated small doses up to a total dose of 6 mg/kg.

Our investigations showed that during hypothermia the enhancing action of analgesics on the interoceptive vascular reflexes is lessened. This effect may be associated with alteration of the functional state of the reflex centers at a low body temperature, due primarily to changes in the functional state of the synapses, which are very sensitive to cooling.

According to Glick [5], Nachmanson et al. [8] and others, during hypothermia changes take place in the cholinesterase and cholinacetylase activity, i.e. the chemical component of synaptic transmission is disturbed. There is no doubt that a change in the reactivity of these enzyme systems is a manifestation of the universal depressing effect of cold on the action of all enzymes, for these are other indications in the literature of the diminished intensity of various enzymic reactions during a fall in the body temperature [3, 6, 7].

The investigations of V. V. Zakusov and his co-workers proved convincingly that the point of application of the action of analgesics is the synapses in the central nervous system (diencephalon [2], spinal cord [1, 4]). Changes in the effects of analgesics on the interoceptive reflexes from the thoracic organs during hypothermia should be regarded as the result of an alteration in the functional state of the central links of the reflex arc, in consequence of the fall in the body temperature.

SUMMARY

The author studied the effect of morphine (2 and 5 mg/kg), promedol (1 and 3,5 mg/kg) and phenadone (1 mg/kg) on the reflex changes of the blood pressure after stimulating the pericardium and the area of the hilum of the lung at normal temperature and in hypothermia (30-32 °C and 25-27 °C).

Experiments were performed on cats. Analgesics intensify the depressor vascular reflexes at normal temperatures; at lower temperatures this action is less pronounced. The change in the analgesic effect on the interoceptive reflexes from the thoracic organs in hypothermia should be regarded as the result of the altered functional condition of the central links in the reflex arc due to lowered body temperatures.

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THE HYPERSENSITIVITY OF RABBITS TO THE TETRACYCLINES

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By comparison with animals showing normal sensitivity to antibiotics, the guinea pig is known to be hypersensitive to penicillin and to the tetracyclines [4-8]. This animal dies from very small doses of these antibiotics when given repeatedly or only once.

There are various theories to account for the mechanism of the toxic action of penicillin and the tetracyclines on guinea pigs. Some authors [5, 7, 8] explain it by the appearance of Escherichia coli, which is not present in animals not receiving antibiotics, in the intestine of the guinea pigs after these antibiotics have been given. Other workers [4, 6] explain the hypersensitivity of guinea pigs by the absence of any penicillin-inactivating substance from the central nervous system of these animals, by allergy to penicillin and so on.

In experiments on guinea pigs (35 animals) weighing from 200 to 400 g, we showed that after the oral administration of antibiotics of the tetracycline group in doses of 10 mg/kg for 2-5 days, Esch. coli and Bacillus proteus were isolated from the intestinal contents of the animals, but were not found in the guinea pigs of the control group.

The guinea pigs which received antibiotics in these doses rapidly lost weight and died in the course of 5-12 days.

The findings of other authors in relation to changes in the intestinal flora of guinea pigs under the influence of the tetracyclines and to the high sensitivity of the animals to these antibiotics were thus confirmed.

Besides the extensive literature on the hypersensitivity of guinea pigs to certain antibiotics, in articles on the treatment of candidomycosis in rabbits, Ambrosioni and Masoni[2, 3] in 1955 noted the high sensitivity of these animals to chlortetracycline. A. M. Kharitonova [1] described the pathological-anatomical changes in rabbits after taking repeated doses of tetracycline.

Since the subject of the sensitivity of rabbits has been inadequately discussed in the literature and is of great importance in the elucidation of the mechanism of the side-effects of the tetracycline antibiotics on the body, we carried out a special investigation in this direction.

EXPERIMENTAL METHOD

Experiments were carried out on 87 rabbits weighing from 1500 to 4100 g. Fifty rabbits were given chlortetracycline, 19-tetracycline and 18-oxytetracycline. The antibiotics were taken by mouth in doses of 5, 25 and 50 mg/kg daily. We used crystalline hydrochlorides of chlortetracycline, tetracycline and oxytetracycline of Soviet manufacture. The animals were weighed daily, and observations were made on their condition. In order to study the changes taking place in the rabbits, periodic investigations were made of their blood, their fecal microflora and, in some cases, their intestinal microflora. Postmortem examination was carried out on 36 rabbits.

Survival of Rabbits After Treatment With Tetracyclines

Dose of drug given (in mg/kg)	Dry chlortetracycline				Dry tetracycline				oxytetracycline			
	total number of experimental animals	number which died	time of death (in days)	loss of weight (in g)	total number of experimental animals	number which died	time of death (in days)	loss of weight (in g)	total number of experimental animals	number which died	time of death (in days)	loss of weight (in g)
	number of animals				number of animals				number of animals			
5	22	22	2—9	50—500	10	10	2—10	100—400	8	8	1—11	50—750
25	4	4	2—5	60—300	5	4 *	2—5	110—370	2	2	8—9	350—380
50	24	23 **	2—31	150—1 000	4	4	2—23	300—1 510	8	8	3—15	100—900

* One rabbit was sacrificed after receiving the drug for 25 days without any signs of toxic effects.

** One rabbit was sacrificed after 33 days in a similar condition.

EXPERIMENTAL RESULTS

Nearly all the experimental animals (83 of 87) receiving the above doses of antibiotics died in the course of two weeks from the beginning of administration of the tetracyclines (see table) with signs of increasing sluggishness, anorexia, loss of weight and profuse diarrhea, with excretion of a large quantity of mucus. Two rabbits died with the same manifestations 15-31 days from the start of administration of the drug. In some rabbits the toxicosis was severe, but there were no intestinal manifestations. Besides the doses indicated in the table, 4 rabbits received chlortetracycline at the rate of 1 and 2 mg/kg, and one rabbit received tetracycline in a dose of 2 mg/kg. All these animals also died, with the signs mentioned above, after 3 to 15 days. It must be pointed out that the trend of the loss of weight in the experimental animals was roughly the same, and depended to a considerable degree on the duration of their survival.

We were unable to establish any essential difference in the clinical picture and the time of death of the animals in relation to the drug and dose given.

The high sensitivity of rabbits to the tetracyclines was thus established with reasonable certainty.

Blood was examined from animals receiving all the above doses of the tetracyclines. The blood analyses were made on fasting animals, at intervals of 2-3 days. The red cells and leucocytes were counted, the hemoglobin level and ESR determined and the leucocyte formula calculated.

Irrespective of the drug they received and the dose, all the rabbits showed a leucocytosis (from 20,000 to 75,000 leucocytes as compared with a normal count of 7,000-10,000) on the 3rd-6th day. The changes in the leucocyte formula consisted of a fall in the number of lymphocytes to 20-30% (normal 50-60%) and an increase in the number of segmented neutrophils to 80% (normal 30-40%). In some animals, on the 2nd-3rd day before death, the ESR was raised to 50-60 mm in 1 hour. The red cell count and hemoglobin concentration were unchanged.

The fecal microflora were investigated in 25 rabbits, receiving tetracyclines in doses of 5, 25 and 50 mg/kg.

The number of E. coli, B. proteus and anaerobic bacteria were counted before the experiment and on the 2nd, 4th, 6th and 8th day (if the rabbits survived so long). In all the experimental animals, irrespective of the drug and the dose given, changes occurred in the number of E. coli and B. proteus.

There was a significant increase in the number of B. proteus. Before the experiment, no B. proteus was found in the feces of the majority of rabbits; in 2 of 25 rabbits a very

small number of these microorganisms was isolated. Starting on the second day of administration of tetracyclines to the rabbits, the number of B. proteus in the feces increased intensively, to reach 10^5-10^9 bacterial cells per g moist feces. At the same time the number of E. coli fell from 10^8-10^9 (before administration of the drug) to 10^3 -10^5 at the end of the experiment; in 2 of 25 rabbits, with intensive growth of B. proteus, only solitary colonies of E. coli were observed. The total number of aerobic bacteria in the majority of animals was increased on account of an increase in the number of B. proteus, but in a few animals it was unchanged. Investigation of the intestinal flora in 8 rabbits gave the same results as investigation of the microflora of the feces. The sharp increase in the number of B. proteus led us to examine the blood for the number of this microorganism contained in it, which might be the cause of death of the animals. Numerous blood cultures from the rabbits at various times were sterile, however.

At postmortem examination of the rabbits dying at different times from the beginning of administration of the drug, a similar pattern of lesions was found. The most marked postmortem changes were seen in the gastrointestinal tract. The stomach was atonic, and in some cases the mucous membrane was ulcerated. In places the loops of small intestine were distended, and elsewhere in spastic contraction. The usual contents of the intestine were not present, but its lumen, especially in the proximal segments, contained a large quantity of thick transparent mucus. In the distended portions, mainly in the distal segments of the intestine, an accumulation of a large quantity of frothy fluid was observed. Congestion of the vessels of the mesentery and serous membrane, mainly of the proximal segments of the intestine, was often noted. The grossest changes were found in the large intestine, especially in the ascending part and the cecum. These portions, especially the cecum, were grossly distended and contained usually a large quantity of flocculent, brownish, frothy fluid. A frequent abnormality was marked congestion of the vessels of the mesentery and serous membrane. In the greater part of the rabbits, the cecum and sometimes the ascending part of the colon showed numerous small, and in some places confluent, hemorrhages beneath the serous membrane. Continuous areas of hemorrhage were also observed beneath the serous membrane of almost the whole extent of the cecum. The mucous membrane of the large intestine remained unchanged macroscopically. Among other changes frequently found may be mentioned considerable congestion of the vessels of the pia mater and liver, less often, congestion of the lungs, and also dystrophic changes of the heart, liver and kidneys.

The changes in the gastrointestinal tract were thus characterized by atony of the stomach, dystonia and catarrhal inflammation of the small intestine, and gross circulatory and functional disturbances of the large intestine. These clinical and morphological changes are the main and most characteristic signs of toxicosis in rabbits developing after administration of antibiotics of the tetracycline group to these animals. The intensity of the morphological changes, from our observations, differed in different animals, but was independent of the type and dose of the drug.

It may be accepted that important factors in the pathogenesis of the changes described in the rabbits are changes in the intestinal microflora and also the direct toxic action of the tetracyclines on the body, and mainly on the gastrointestinal tract.

SUMMARY

It has been established that rabbits possess an increased sensitivity to antibiotics of the tetracycline group. On administering chlortetracycline, tetracycline, or oxytetracycline to rabbits per os in doses of from 5 to 50 mg/kg per day the majority of the animals died within 2 weeks after initiation of the treatment, with phenomena of increasing apathy, anorexia, weight reduction and profuse diarrhea.

Large numbers of B. proteus appeared in the intestinal contents of rabbits which received tetracyclines.

Leukocytosis was noted in the blood due to the increase in the number of polymorphonuclear segmented neutrophils and relative lymphocytopenia was also present.

The most pronounced pathological-anatomical changes resulting from tetracycline administration to rabbits were observed in the gastrointestinal tract.

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EXPERIMENTAL DATA ON THE EFFECT OF PHTHIVAZID AND TUBAZID ON THE BODY

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In addition to their bacteriostatic action on Mycobacterium tuberculosis, phythivazid• and tubazid•• also have a definite pharmacodynamic effect on the body as a whole. In the foreign literature, for instance, there are reports that tubazid and certain other derivatives of isonicotinic acid have an influence on the autonomic nervous system, exerting a sympatheticomimetic action [12, 17].

There are many conflicting experimental and clinical findings in connection with the effect of isonicotinic acid preparations on carbohydrate metabolism, but the majority of these suggest that under the influence of various nicotinic acid preparations the blood sugar level rises and diabetic hyperglycemia is increased [13, 16 and others]. Certain workers have attempted to explain the hyperglycemic effect of these preparations by increased mobilization of glycogen from the liver and muscles, but no direct evidence in favor of this is produced [20]. The deleterious effect of isonicotinic acid on the liver has been shown experimentally [16, 15, 21 and others] and confirmed by clinical observations [14].

Our previous studies of the action of phthivazid on the neuro-humoral changes in the blood showed that the use of this drug was accompanied by normalization of the disturbances of the mediator system observed in patients with tuberculosis. So far as the effect of phthivazid on carbohydrate metabolism is concerned, experimental findings and clinical observations indicate that it has a weak insulin-like action [7, 10]. The positive action of phthivazid on liver function has been demonstrated both experimentally and clinically [2, 6, 7 and others].

We undertook the task of comparing the effects of phthivazid and tubazid on certain interconnected metabolism processes in the body, the reactions of which are to a large extent determined by the functional state of the autonomic division of the central nervous system.

Our previous investigations showed that in healthy rabbits and in rabbits inoculated with tuberculosis, receiving phthivazid by mouth for 4-5 weeks, the liver glycogen content was considerably higher than in healthy and tuberculous animals not receiving phthivazid. Besides an accumulation of glycogen in the liver, a fall in the lipid content was observed in the healthy animals (in animals receiving phthivazid – 11.4g% of lipids, in healthy controls – 17 g%).

In rabbits taking phthivazid by mouth we observed a lower degree of accumulation of fat in the liver during

• Isonicotinoyl- (3-methoxy-4-oxybenzal) hydrazone.– Translator.

•• Isoniazid. – Translator.

Mean Values of the Lipid Content in the Liver and the Glycogen Content in the Liver and Muscles of Control Rabbits and of Rabbits Receiving Phthivazid and Tubazid

Number of animals	Control rabbits		Rabbits received phthivazid			Rabbits received tubazid			
	liver glycogen	liver lipids	Number of experiments	liver glycogen	liver lipids	Number of experiments	liver glycogen	muscle glycogen (in g%)	liver lipids
15	2,5 (2,0—4,0)	17,0 (12,7—21,0)	15	4,2 (3,3—4,8)	15,0 (11,0—17,5)	15	1,8 (0,8—3,0)	0,6 (0,4—0,8)	19,8 (15,0—24,0)

Note. The limits of variation are given in brackets.

fatty infiltration due to carbon tetrachloride. These results are in agreement with those of N. P. Skakun and L. P. Turko [7], who obtained good therapeutic results from the use of phthivazid in dogs with experimental hepatitis due to carbon tetrachloride. The favorable effects of phthivazid on certain liver functions, shown experimentally, provide a basis for the use of phthivazid in the treatment of a number of diseases of the liver [11].

EXPERIMENTAL METHOD

In the present communication we described the results of investigations carried out on 3 groups of rabbits (15 animals in each group), weighing about 2 kg, and kept under identical conditions of diet and general care. One group received phthivazid in a dose of 100 mg/ kg body weight by mouth for 5-6 weeks, the second group received tubazid (10 mg/ kg body weight) for the same length of time and the third group acted as control animals.

Investigations were carried out before the drug was given, 2 weeks after it had been started, and then at the end of its administration, i.e., after 5-6 weeks. Some animals were examined once more, 4-5 days after the end of administration of the drug. At the same time the chemical factors of nervous excitation in the blood were estimated. These estimations were not only made at at the time mentioned above but also 4 hours after the first administration of the nervous excitation preparation, since we know from the investigations of V. A. Buskina, L. I. Grebennik and E. É. Fialko [1, 3, 9] that the maximum concentration of phthivazid in the blood is noted 2-4 hours after its administration.

The animals were killed by air embolism 5-6 weeks after receiving phthivazid and tubazid, and estimations were made of the lipid content (by Leites' method) and the glycogen content (by Pflüger's method) of the liver. Because of reports in the literature of the possible mobilization of glycogen from the muscles under the influence of isoniazid, we also investigated the muscle glycogen in the rabbits receiving tubazid.

The mediators of the blood were tested on biological objects: cholinergic drugs on the denervated dorsal muscle of the leech (after preliminary treatment with proserine) and on the isolated frog's heart. Blood for testing was taken from the auricular vein of the rabbit and stabilized with synanthrol. In order to prevent decomposition of the unstable acetycholine, the blood was collected in a syringe which contained a small quantity of proserine as a cholinesterase inhibitor.

EXPERIMENTAL RESULTS

The study of the neuro-humoral changes in the blood of healthy rabbits, before receiving phthivazid, showed that the blood of these animals did not cause contraction of the denervated dorsal muscle of the leech and had no negative ino-chronotropic effect on the isolated frog's heart. The first examination of the rabbits 4 hours after the start of phthivazid administration showed, however, that the blood of the majority of the experimental animals contained a biologically active substance, evidently parasympathetic

in origin, for it caused contraction of the isolated dorsal muscle of the leech. Examination of these same rabbits throughout the experiment (after 2 weeks, and then at the end of phthivazid feeding) revealed that the parasympatheticomimetic effect of the blood was the more apparent the longer the period of action of the drug on the animal.

Investigations on the sugar concentration in the peripheral blood of these animals showed that its level varied between normal limits (from 70 to 100 mg%) in the individual animals throughout the period of administration of the drug; this agrees fully with investigations on patients receiving phthivazid.

The prolonged administration of tubazid led to different changes in the experimental animals. Tubazid brought about sympatheticomimetic changes in the mediator system, which were clearly seen 2 weeks after the start of its administration and reached very high figures at the end of the experiment (the positive inotropic action of the blood at the end of administration of the drug varied from 120 to 200% the blood of the control animals had an insignificantly positive inotropic effect of from 10 to 42%).

In all the animals receiving tubazid the blood sugar rose, and in some of them it reached 125-135 mg%, as compared with an initial level of 70-90 mg%. The mean increase in the blood sugar for the whole group of rabbits over its initial concentration before receiving tubazid was 35 mg%. The increase in the blood sugar level was proportional to the duration of administration of the drug: for example, estimations of the blood sugar 5 days after administration had ended showed that the hyperglycemic effect of tubazid was unstable and lasted only while the drug was given.

The effect of phthivazid and tubazid on the lipid and glycogen content of the liver also differed (see table)*.

It will be seen from the table that the glycogen content of the liver in rabbits receiving phthivazid was much higher than that in rabbits receiving tubazid, and higher still than that in the control animals, according to both the mean value and the limits of variation.

The small decrease in the glycogen content of the muscles in rabbits receiving tubazid may possibly be due to mobilization of glycogen from the muscles and to intensification of the processes of glycogenolysis in both muscles and liver, which lead to a hyperglycemic effect

In order to exclude the direct action of phthivazid and tubazid on the blood, we made a series of observations: the drugs were added to 5 ml of whole blood from a rabbit (in a test-tube) in a concentration equivalent to that in the blood of the experimental animals during administration of phthivazid or tubazid (phthivazid — 4-7 γ/ml, tubazid — 6-8 γ/ml). This blood was investigated in the usual manner on biological test objects. It was found that the blood so tested did not cause contraction of the proserinized dorsal muscle of the leech and had no effect on the isolated frog's heart, no matter whether phthivazid or tubazid were added to it.

The parasympatheticomimetic effect of phthivazid and the sympatheticomimetic effect of tubazid thus did not depend on the direct action of these drugs on the blood; these effects must evidently be due to the complex changes developing in the animal as a result of the administration of these drugs.

It may be concluded from the results obtained that phthivazid and tubazid act differently on certain physiological functions of the body. Phthivazid, for instance, when giving by mouth, leads to the development of parasympatheticomimetic changes in the mediator systems of healthy animals, does not alter the sugar content of the blood when its initial level is normal and possesses a glycogen-forming action, while at the same time restricting the accumulation of fat in the liver.

In these conditions, tubazid causes a well marked sympatheticomimetic effect and also raises the blood sugar, creating in individual rabbits a hyperglycemic effect which increases with the duration of administration of the drug, but is unstable and disappears on stopping the drug. Prolonged administration of tubazid lowers the glycogen content of the muscles and liver and leads to accumulation of a large amount of fat in the liver. This action of tubazid on the glycogen and fat contents of the liver may possibly partially explain the deleterious effect of the drug on liver function, which is observed clinically.

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*The experimental results were treated statistically and the mean square deviation and the mean error determined, the cube of which did not exceed the mean value found. The arithmetic means obtained were thus significant.

The difference in the action of these two drugs on certain functions of the body suggests that phthivazid is superior to tubazid in its more sparing physiological action. In the choice of antibacterial drugs, clinicians must be guided by the differences in the action of these on the various functions of the body.

SUMMARY

The author studied the effect of phthivazid and tubazid on some physiological functions of rabbits.

It was found that phthivazid, administered per os to healthy animals for a long period of time, promotes parasympathomimetic shifts in the mediator system, has no effect on the blood sugar leve, and possesses a glycogen-forming effect, thus evidently limiting the accumulation of fat in the liver.

Under the same conditions tubazid evokes a pronounced sympathomimetic effect, increases the blood sugar level, reduces the glycogen content in the muscles and liver, and promotes fat accumulation in the liver.

The difference in the action of these two preparations on the body suggests that phthivazid has an advantage over tubazid due to its more sparing physiological effect.

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THE EFFECT OF DIMEDROL* ON THE DEVELOPMENT OF TUBERCULIN REACTIONS

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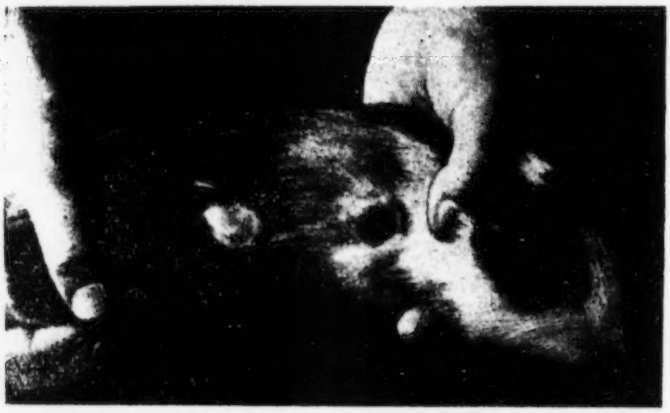
The role of histamine in the pathogenesis of anaphylactic shock was confirmed by experiments in which this type of shock was prevented by means of antihistamine drugs. Since histamine is thought to play a definite part in the pathogenesis of allergic reactions in tuberculosis, attempts were made to prevent the development of tuberculin reactions also by means of antihistamine drugs [3, 4]. The results obtained however, were not very illuminating.



Development of the tuberculin reaction in a guinea pig. From left to right: 1st area of skin – hyperemia of the skin (tuberculin + dimedrol injected); 2nd area of skin – hemorrhages in the skin (tuberculin alone injected).

EXPERIMENTAL METHOD AND RESULTS

In order to study the action of dimedrol we gave subcutaneous inoculations with 0.001 mg of a Valle culture to 30 guinea pigs, and used these animals for further investigations during the 5th week. In all, 38 experiments were performed (92 tests) on the 30 guinea pigs.

The hair was removed at two points on the lateral abdominal wall of the guinea pigs; at one of these points, 0.1 ml of tuberculin, diluted with ten times its volume of dimedrol (1:10 000), was injected into the skin, and at the other, 0.1 ml of tuberculin, diluted 1:10 with physiological saline, was injected.

* = diphenhydramine hydrochloride (Benadryl) (Translator's note)

Tuberculous guinea pigs are known to react in two ways to the intradermal injection of tuberculin: either by erythema and infiltration at the site of injection of the tuberculin or by a hemorrhagic reaction and infiltration.

It was shown that if the tuberculous guinea pigs reacted to tuberculin (in the control area) by a hemorrhagic reaction, then under the influence of dimedrol the hemorrhagic reaction in the test area was completely abolished. If the guinea pig reacted to tuberculin in the experimental area by erythema and infiltration only, then dimedrol had no effect whatsoever on this reaction.

These findings are in agreement with those which we obtained in experiments on the prevention of the Schwartzmann phenomenon by means of dimedrol [2].

In no case did dimedrol abolish the tuberculin reactions completely, but it prevented the development of hemorrhagic reactions in the skin.

SUMMARY

Two types of reactions are produced in testing the sensitivity of tuberculous guinea pigs to tuberculin — either erythematous or hemorrhagic.

Dimedrol removes only the hemorrhagic component.

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MICROBIOLOGY AND IMMUNOLOGY

THE EXPERIMENTAL PRODUCTION OF VACCINE STRAINS OF SHIGELLA FLEXNERI AND THE STUDY OF THE IMMUNOLOGICAL EFFECTIVENESS OF A LIVING DYSENTERY VACCINE

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The problem of obtaining living vaccines for the specific prophylaxis of enteric infections continues to be one of urgent importance. Great importance in this respect is attached to work on the development of a living dysentery vaccine [16, 19].

In the present paper we describe the results of four years' (1954-57) experimental research on the production of vaccine strains of Shigella flexneri and on the investigation of the properties of the living dysentery vaccine [4, 5].

TABLE 1

The Principal Biological Properties of the Test Strains

Serial No.	Identification No. of strain of Shigella flexneri	Whence isolated	Date of isolation	Virulence LD^{50} (in 10^6)	Antigenicity in %	Toxicity, LD^{50} (in 10^9)	Titer of agglutination with specific serum
1	No. 6267, type c	From stools	10/22/52	250	86	4	1:12 800+++
2	No. 6211, type f	"	10/17/53	250	75	4	1:12 800+++
3	No. 266, type a	"	2/15/50	250	80	2	1:12 800+++

EXPERIMENTAL METHOD AND RESULTS

Three strains of Shigella flexneri — No. 6267 type c, No. 6211 type f and No. 266 type a — were used in the experiments. The first two were obtained from the patients with acute dysentery and the last was a production strain obtained from the government control institute. Our aim was to obtain a considerable and lasting fall in the virulence of these strains while preserving their antigenicity and other biological properties.

For this purpose we carried out experiments on the cultivation of Shigella flexneri in bile in conjunction with passage through cold-blooded animals insusceptible to dysentery. Bile is known to stimulate the growth of a number of microorganisms and to stabilize their newly acquired properties. Repeated subculture in bile broth

TABLE 2. Characteristics of the Principal Properties after 10 Passages through the Frog's Brain

Serial no.	Strain no.	Morphological and staining properties	Biochemical properties	Form of dissociation	Titer of agglutination	Virulence (in 10^9)	Antigenicity (in %)
1	6267	Typical	Typical	S	1 : 12 800	5	86
2	6211	"	"	»	1 : 12 800	1	65
3	266	"	"	»	1 : 12 800	1	80

of the microorganisms of anthrax, swine erysipelas and other diseases brings about a sharp reduction in their virulence and stable preservation of newly acquired properties [17]. When Shingella flexneri is grown in 20% bile broth, a sharp decrease in its virulence is observed [10].

The use of cold-blooded animals for the experimental cultivagion of dysentery microorganisms was established long ago. In 1884, I. I. Mechnikov observed that the virulence of Bacillus anthracis was greatly diminished by injection under the skin of frogs. When toads (Bufo vulgaris) were inoculated with Bacillus pestis, it was found [3] that the longer the organism existed in the body of the cold-blooded animals, the greater was the decrease in its virulence. Similar experiments with Bacterium tularense also confirmed the possibility of lowering the virulence of these microorganisms [12].

The dysentery strains tested all possessed characteristic biological signs (Table 1).

All the properties were assessed by the generallly accepted method. The virulence and toxicity were estimated by the value of the LD_{50}, the results obtained being treated by the method of Read and Mench.

It will be seen from Table 1 that the strains possessed high virulence and antigenicity and low toxicity and were agglutinated to a titer of 1:12 800. Also, they were present in the S-form, they possessed high specificity and were phagolytic.

In the first series of experiments the optimal concentration of bile-peptone broth and cultivation time were selected. As a result several important factors were elucidated. The greatest fall in virulence took place during cultivation at constant temperature (37°) in 60% bile-peptone broth. From the LD_{50} values, the virulence of strain No. 6267 was 5×10^9/ml, and that of strains Nos. 6211 and 266 was 750×10^6/ml. The antigenicity remained high (between 60 and 80%), even after cultivation for 120 days in 60% bile-peptone broth. The remaining biological properties, namely agglutinability, specificity, form of dissociation, saccharolytic activity, etc. remained unchanged. It was remarkable that the cultivation of Shigella flexneri in bile-broth brought about a decrease in virulence but usually stabilized all the remaining properties typical of this species of microoganism.

However, the level to which the virulence of strains Nos. 6211 and 266 had been lowered was still too high, and furthermore, after passage through white mice, the virulence reappeared. We therefore decided to cultivate the microorganisms in bile-peptone broth and to combine this with cultivation in cold-blooded animals. These experiments were performed on pond frogs (Rana radibunda), which were inoculated intracerebrally. From our point of view, this method of introduction of the infective material had two advantages: firstly, the usual medium for its existence in an insusceptible animals must promote a further lowering of the virulence of the microorganisms, and secondly, it would be easier to obtain a pure culture, uncontaminated by other microorganisms, from the frog's brain.

The culture was injected into the midbrain in the region of the posterior third of the parietal bones. A 24-hour culture, suspended in physiological saline, containing 10^9 bacterial cells per ml was used for inoculation. The volume of the suspension injected was 0.05 ml. For passage, 48 hours after inoculation, the frog's skull was opened, the brain extracted and seeded with a pure dysentery culture which, after thorough testing, was again injected into the brain of a frog. Ten passages were performed.

Table 2 shows the results of tests of the strains after the last passage, indicating that they preserved their

TABLE 3

Changes in the Virulence and Antigenicity after Cultivation in a Medium Containing Bile and Passage through the Frog's Brain

Serial no.	Strain no.	1st passage				2nd passage				3rd passage			
		60% bile broth		frog's brain		60% bile broth		frog's brain		60% bile broth		frog's brain	
		virulence LD_{50} (in 10^9)	antigenicity (in %)	virulence LD_{50} (in 10^9)	antigenicity (in %)	virulence LD_{50} (in 10^9)	antigenicity (in %)	virulence LD_{50} (in 10^9)	antigenicity (in %)	virulence LD_{50} (in 10^9)	antigenicity (in %)	virulence LD_{50} (in 10^9)	antigenicity (in %)
1	6207	5,0	80	5	80	5	80	5	89	5	87	5,5	85
2	6211	4,5	73	5	70	4	70	4	79	4	76	5,0	75
3	266	5,0	71	5	71	4	73	4	88	5	76	5,0	78

TABLE 4

Changes in the Virulence and Antigenic Properties of "Reversion" Strains of Shigella flexneri

Serial no.	Strain no.	Total number of subcultures	Duration of subcultures	1955		1956				1957			
				Quarters									
				III	IV	I	II	III	IV	I	II	III	IV
1	6267	654	2 1/2 years	4×10^9 / 80%	5×10^9 / 84%	5×10^9 / 88%	$4,5\times10^9$ / 86%	4×10^9 / 85%	5×10^9 / 86%	5×10^9 / 85%	4×10^9 / 84%	4×10^9 / 85%	$3,5\times10^9$ / 80%
2	6211	304	1 year						3×10^9 / 75%	3×10^9 / 72%	2×10^9 / 70%	2×10^9 / 75%	3×10^9 / 70%
3	266	654	2 1/2 years	4×10^9 / 82%	4×10^9 / 80%	4×10^9 / 88%	4×10^9 / 75%	4×10^9 / 82%	3×10^9 / 70%	3×10^9 / 72%	4×10^9 / 77%	3×10^9 / 80%	3×10^9 / 75%

Note. Legend: numerator — virulence as shown by the value of LD_{50}; denominator — antigenicity by survival rate of white mice.

TABLE 5. Immunobiological Changes in Kittens after Immunization and Infection (Mean Values)

Serial no.	Description of material	Agglutination reaction				Phagocytic reaction			
		before experiment	after vaccination			before experiment	after vaccination		
			9th day	14th day	22nd day		9th day	14th day	22nd day
1	Living vaccine	Negative	1:400	1:400	1:200	9 / 11	24 / 31	30 / 37	27 / 36
	Heat-treated	The same	1:62	1:62	1:50	10 / 12	20 / 25	22 / 24	16 / 21
3	Mixture of virulent initial strains	Negative	1:270	1:220	1:200	7 / 10	27 / 31	32 / 36	33 / 40

Note. Legend: numerator – specific phagocytic reaction; denominator – nonspecific phagocytic reaction.)

TABLE 6. Properties of Living Dysentery Vaccine at Different Periods of Storage

Strains composing the vaccine	Original properties			1st quarter			2nd quarter			3rd quarter		
	virulence LD_{50} (in 10^9)	antigenicity (in %)	toxicity LD_{50} (in 10^9)	virulence LD_{50} (in 10^9)	antigenicity (in %)	toxicity LD_{50} (in 10^9)	virulence LD_{50} (in 10^9)	antigenicity (in %)	toxicity LD_{50} (in 10^9)	virulence LD_{50} (in 10^9)	antigenicity (in %)	toxicity LD_{50} (in 10^9)
6267, type c 6211, type f 266, type a	5	87	4	5	85	4	4	80	3,7	3	80	3,5

principal typical properties, and primarily their antigenicity, although the values of the LD_{50} for strains 6211 and 266, a measure of their degree of virulence, still remained in the region of 10^9. In order to obtain a greater lowering of virulence we studied the possible periods of keeping the culture in the frog's brain. All these strains were kept in the brain for 20, 25, 30, 40 and 45 days.

After 20 days the virulence of strain 266 showed a considerable fall, as shown by the value of LD_{50} – 5 x 10^9. After 30 days the virulence of strain 6211 also reached these figures. So far as the effect of the period of keeping on the properties of the strains is concerned, they still divided actively after 30 days, and preserved their principal typical properties. At still later periods, some dying away of the microorganisms took place, although they continued to divide.

In the next series we undertook serial cultivation of strains in bile-peptone broth and frog's brain. We named each passage in turn, and tested three such passages. In each passage, cultivation was in 60% bile-peptone broth for 30 days; in the first passage the culture was kept in the frog's brain for 45 days and in later passages 30 days. After each passage complete testing of the culture was carried out.

The results in Table 3 reveal usually stable indices of virulence and antigenicity in the course of the passages. According to these indices, all three strains could be described as highly antigenic and with virulence equivalent to an LD_{50} value of 4 and 5 x 10^9. Full testing of the remaining biological properties satisfied us that they preserved the typical signs of this species of microorganism.

Systematic experiments in possible reversion of virulence were conducted by seeding all three strains on solid and liquid nutrient media.

Reversion of virulence experiments on strain 6267 were started in August and those on strain 266 in September 1955, those on strain 6211 in September 1956. The cultures were seeded on Petri dishes with weakly alkaline agar. After 18-20 hours S-form colonies were picked off the dishes, checked by the trypaflavine reaction and subcultured on agar slopes in test tubes. The biological properties of the 24-hour cultures on agar slopes were studied. We know from the literature that frequent subcultures of Shigella flexneri usually react in dissociation to R-forms and to loss not only of virulence but of antigenic properties also [7, 9, 13].

Virulence and antigenicity trails of the serial subcultures of the reversion strains demonstrated the usual stability of these properties (Table 4). All three strains retained their typical morphology, were agglutinated by specific serum to a limiting titer (1:12 800), were present in the S-form of dissociation, retained their typical biochemical activity and were not agglutinated by heterogenic sera, i.e., they remained specific.

The next series of experiments was devoted to the production of a living dysentery vaccine from the vaccine strains studied, and to the testing of its properties. Several series of vaccine were prepared and two were studied in detail: the first, prepared in December 1955, was from two strains — 6267 and 266, and the second, prepared in December 1956, was from three strains — 6267, 6211 and 266. The vaccine was prepared by washing a 24-hour culture of the microorganisms with physiological saline. A standard for the suspension was established at 4×10^9/ml. The usual method of quality control were employed. The vaccine selected for the experiment had the typical morphology and antigenicity, low toxicity and virulence equivalent to an LD_{50} value of 4 x to 5 x 10^9. The antigenic properties of the vaccine were further determined in experiments on kittens. A large volume of information has now been accumulated on the possibility of reproducing dysentery infection in kittens and adult animals [1, 2, 11, 14, 15, 18].

In our experiments 15 kittens were used in trails of the vaccine prepared from two strains (6267 and 266). Natural infection of the animals with the Shigella and Salmonella groups of microorganisms was first excluded, as also was the presence of immunity. The animals were divided into three groups: the 1st group (6 kittens) was immunized with living dysentery vaccine, the 2nd group (4 kittens) — with heat-treated vaccine (prepared from the original strains and killed by heating) and the 3rd group (5 kittens) received the same dose of a mixture of the original virulent strains. The animals were immunized and infected once only, by mouth, after being starved for 24 hours, being given 4×10^9 bacterial cells suspended in 2 ml of warm milk.

The experiments showed that the control group of kittens became ill 4-6 days after infection, with characteristic clinical manifestations — dyspeptic disorders, raised temperature, adynamia and other signs [3, 4]. No clinical manifestations of the disease were observed in the kittens receiving living and heat-treatment vaccines.

The immunobiological changes as a result of immunization and infection were tested with serum from the experimental animals by means of the agglutination and phagocytic reactions. The phagocytic activity of the leucocytes was studied. So that the trend of these changes could be followed, the rezctions were carried out on different days after administration of the material.

The results in Table 5 show that such a weak immunizing stimulus as a single oral immunization with living vaccine caused a marked accumulation of antibodies on the 9th-14th day after administration of the material. The antibody titer after immunization with heat-treated vaccine lagged behind considerably. Similar features could be observed on analysis of the trend of both the specific and the nonspecific opsonophagocytic reaction.

Experiments on vaccination of kittens with living dysentery vaccine were repeated in 1957. Twenty-four animals were used, divided into two equal groups: one of these was vaccinated and the second infected with a mixture of original virulent strains. The preparation tested was a living vaccine prepared from three strains (6267, 6211 and 266) in December 1956.

Immunobiological changes in the two groups of animals were tested alone the same lines. As additional measures, the complement fixation reactions was carried out with serum from the immunized animals, and the hapten reaction with the animals' feces. The most delicate and earliest test was the complement fixation reaction, which was positive only 48 hours after vaccination. Diagnostic titers in the agglutination reaction were observed on the 6th day, and at the same time the hapten reaction also became positive. A marked increase in

the phagocyte activity of the leucocytes was observed after 72 hours, and this increased progressively in the subsequent tests. The immunobiological changes in the immunized kittens were no less pronounced than those in the infected animals.

The properties of the last vaccine were tested at intervals during storage in the refrigerator for 9 months.

The results in Table 6 show that during the period of observation the vaccine remained avirulent, nontoxic, and highly antigenic. Monthly testing of the survival of the vaccine, carried out by the method used for assay of living plague vaccine, showed that it varied between limits of 50% (after storage for 1 month) and 30% (Storage for 9 months).

SUMMARY

As a result of observations conducted for many years, the authors have developed a method of obtaining avirulent and high immunogenic strains of Shigella flexneri. The principle of this method consists of cultivating the microorganisms in cold-blooded animals (pond frogs) resistant to this infection, as well as on animal products (60% bile-peptone broth).

An usual stability of the acquired properties, which is not characteristic for this species of bacteria, was established. 3 experimental strains appeared to be avirulent, and highly immunogenic and retained all the biological properties. The possibility of studying dysentery immunization problems on young cats was demonstrated.

Living Shigella flexneri vaccine possesses immunological efficacy in experiments on this species of animals; it may be stored in a refrigerator up to 9 months without losing its properties.

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ONCOLOGY

THE ELECTROPHYSIOLOGICAL ANALYSIS OF DISTURBANCES IN THE TROPHIC ACTION OF THE NERVOUS SYSTEM DURING THE DEVELOPMENT OF A MALIGNANT TUMOR

(BASED ON THE RESULTS OF ANIMAL EXPERIMENTS)

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It has been shown by clinico-physiological observations on patients suffering from carcinoma, polyposis or ulcer of the stomach that the development of malignant tumors is associated with disturbance of the neuro-humoral regulation, as a result of a lowering of the tone of the sympathetico-adrenal system [1-4].

By way of studying in greater detail the character of the changes taking place in the various divisions of the nervous system during tumor development, and of ascertaining the importance of the tone of the sympathetic nervous system to the rapidity of development of the tumor in the body, we carried out certain investigations, the results of which are described in this article.

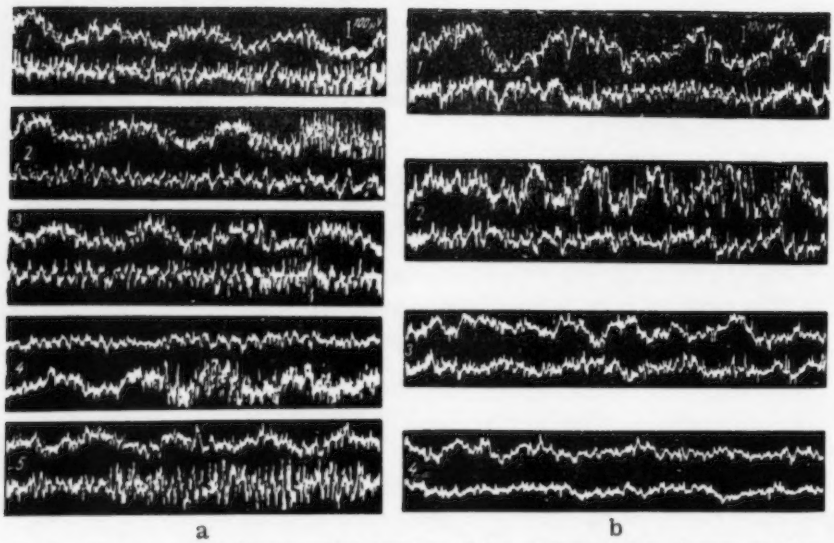
EXPERIMENTAL METHOD

Electrophysiological experiments were carried out on rabbits in which a Brown-Pearce tumor had been transplanted. Before transplanting of the tumor, unipolar and bipolar electrodes were introduced into different divisions of the central nervous system. In chronic and acute experiments stimultaneous recordings were made of the so-called spontaneous bioelectrical activity of the cerebral cortex, the hypothalamus and the cerebellum during the period of development of the tumor and until the animal's death, using a multi-channel oscillograph. In acute experiments we also recorded the bioelectrical activity of the superior cervical sympathetic ganglia.

EXPERIMENTAL RESULTS

In the chronic experiments, during which the bioelectrical activity of the various divisions of the nervous system was continuously recorded in 14 rabbits, it was found that on the 7th-11th day after transplantation of the tumor there was a sharp increase in the bioelectrical activity of the cerebral cortex. This took the form of an increase in the amplitude and frequency of the slow and rapid waves of the electroencephalogram. The bioelectrical activity of the cerebral cortex and hypothalamus is simultaneously recorded on the separate strips in Fig. 1 a and b, which are in sequence corresponding with the growth of the tumor. The increase in the bioelectrical activity of the cerebral cortex, as we were able to observe subsequently, was found to have a reflex effect a short time afterwards on the centers of the hypothalamus (see Fig. 1, a, 1 and 3) and also of the cerebellum (Fig. 2, a, 1 and 2) and the sympathetic ganglia (see Fig. 2, b, 1 and 2).

The initial increase in the bioelectrical activity of the nerve centers in the rabbits was connected with the effect of impulses reaching the central nervous system from the interoceptors in the course of development of the tumor and multiple metastases. We showed that procaine when injected in the region of the tumor and



a b

Fig. 1. Changes in the bioelectrical activity of the cerebral cortex (upper tracing on the strips) and hypothalamus of a rabbit (lower tracing on the strips) during the development of a Brown-Pearce tumor (a) in a rabbit with increased reactivity of the hypothalamus and (b) in a rabbit with diminished reactivity. In the case of (a): 1) bioelectrical activity of the cortex and hypothalamus before transplanting of the tumor; 2) on the 11th day after transplantation of the tumor; 3) on the 15th day after transplantation of the tumor; 4) on the 23rd day after transplantation of the tumor; 5) on the 26th day after transplantation of the tumor. Time marker – 1/20 second (shown above one strip for all the electroencephalograms). In the case of (b): 1) bioelectrical activity of the cerebral cortex and hypothalamus before transplantation of the tumor; 2) on the 15th day after transplantation of the tumor; 3) on the 21st day after transplantation of the tumor; 4) on the 23rd day after transplantation of the tumor. Time marker as in Fig. 1, a.

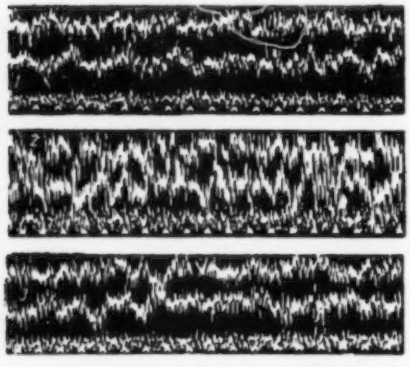


Fig. 2a. Action potentials of the cerebral cortex, hypothalamus and cerebellum. 1) before transplantation of a Brown-Pearce tumor; 2) on the 14th day after transplantation of the tumor; 3) after the action of procaine (on the 14th day after transplantation of the tumor).

retroperitoneally (10 ml of a 0.25% solution) caused a sharp fall in the bioelectrical activity of the centers (Fig. 2, a, 3). After the effect of the procaine had worn off (in 30-40 minutes) the excitation of the centers was usually restored.

During the subsequent development of the tumor, on the 20th-25th day from the moment of transplantation, the bioelectrical activity of the cerebral cortex began to fall gradually until the time of death of the animals, but not in all of them. As we were able to show, all the changes in the functional state of the cerebral cortex taking place after the initial rise in its excitation were closely connected with the character of the changes developing reflexly in the functional state of the autonomic centers, and in particular in the hypothalamus and the sympathetic ganglia. In those cases when the state of excitation developing reflexly in the hypothalamus was considerable in degree and stable by comparsion with the initial background – there were 3 such animals among the 14 in this series (see Fig. 1, a, 3-5) –, the level of excitation of the nerve cells of the cere-

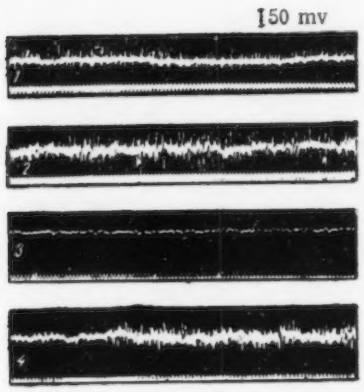


Fig. 2b. Character of bioelectrical activity of the superior cervical sympathetic ganglion of the rabbit. 1) before transplantation of a Brown-Pearce tumor; 2) bioelectrical activitity of the ganglion 10 days after transplantation of the tumor; 3) bioelectrical activity of the ganglion in the presence of a developing tumor with multiple metastases in the abdominal region; 4) recovery of the bioelectrical activity of the ganglion as the result of the action of combined drugs. Time marker – 1 second (below – 1/60 sec).

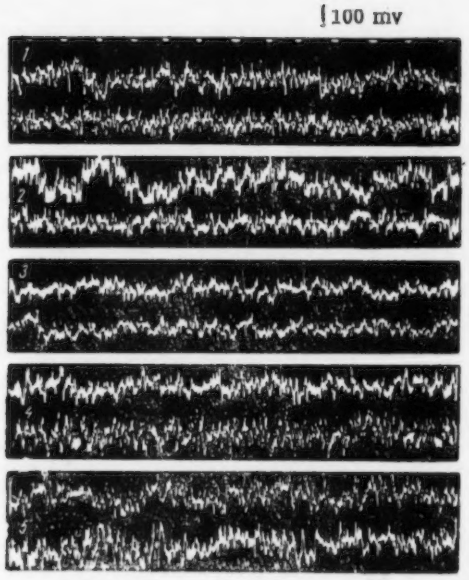


Fig. 3. The stimulating effect of a drug combination on the bioelectrical activity of the cerebral cortex (upper tracing on the strips) and of the hypothalamus (lower tracing on the strips) of a rabbit, diminished by the presence of a Brown-Pearce tumor. 1) bioelectrical activity of the cerebral cortex and hypothalamus before transplantation of the tumor; 2) on the 11th day after transplantation of the tumor; 3) on the 15th day after transplantation of the tumor; 4) on the 23rd day after transplantation of the tumor; 5) on the 30th day after transplantation of the tumor. Time marker – 1/20 second (shown above one strip for all the electroencephalograms).

bral cortex increased correspondingly (see Fig. 1, a, 5), for before this they were gradually falling under the influence of prolonged afferent impulses from the periphery and, after their initial excitation, were in a state of lowest activity (see Fig. 1, a, 4).

Tests of the bioelectrical activity of the superior cervical sympathetic ganglia of the rabbit in acute experiments (on the 27th day after transplantation of the tumor) showed that the synaptic transmission of impulses in the ganglia continued to be preserved; occasionally an increase in the impulsation could even be observed, analogous to that shown in Fig. 2, b, 2.

The increased activity of the hypothalamus in a number of rabbits was connected with high lability and reactivity of the nerve cells of the hypothalamus. This could be judged by the periodic sharp bursts of "spontaneous" bioelectrical activity observed under normal conditions, before transplantation of the tumor (see Fig. 1, a, 1, at the end).

In rabbits with a lesser degree of lability of the nerve cells of the hypothalamus, in response to the sharp increase in the bioelectrical activity of the cerebral cortex after transplantation of the tumor, a depression of hypothalamic activity took place in corsequence of the reflexly induced brief burst of excitation; this may be judged by the reduction in the amplitude of the potentials (see Fig. 1, b, 4). Aganist this background, the initial excitation of the cerebral cortex of the rabbits was later (on the 15th-28th day from the moment of transplantation of the tumor) replaced by a fall in the level of the bioelectrical activity, which took the form of a sharp

reduction in the amplitude of both the slow and fast waves of the electroencephalogram. Tests of the bioelectrical activity of the superior cervical sympathetic ganglia in an acute experiment on the same rabbit (on the 23rd day after transplantation of the tumor) showed that the synaptic transmission of the impulses in the ganglia was practically absent. As a result, inhibition had then developed in the peripheral division of the sympathetic nervous system (see Fig. 2b, 3). We also studied the functional state of the sympathetic ganglia in rabbits during growth of the tumor in another series of acute experiments in which the bioelectrical activity of the ganglia was recorded on different days after transplantation of the tumor (10 rabbits); a disturbance of the transmission in the ganglia of the sympathetic nervous system, in which inhibition had developed, always preceded deterioration in the state of the rabbits.

The electrophysiological data obtained gave evidence of a direct interconnection between the functional state of the various divisions of the nervous system. The character of the reactivity of the hypothalamic centers, and also the functional state of the cells of the sympathetic ganglia(through which the central nervous system is known to send impulses for mobilization of the energy resources of the body) are directly concerned in the maintenace of the functional state of the cells of the cerebral cortex at a definite level. thereby ensuring the general regulation of the trophism of the tissues of the central nervous system.

As the experiments showed, the character of the relationship between the processes of excitation and inhibition on the cerebral cortex, the hypothalamus and the sympathetic ganglia determined the direction of development of the pathological process taking place in the animal. With increased excitation of the centers of the autonomic nervous system (and, in particular, of the hypothalamus and the sympathetic ganglia, through which a continous flow of impulses passes to the periphery), the neuro-humoral regulation of the tissues was maintained at a corresponding level, and the body took active measures to fight against the malignant tumor. After the initial growth of the tumor, in some cases it was observed to undergo absorption. At autopsy on one of the animals of this group on the 27th day an almost complete absence of metastases was observed. In two rabbits, after 2 months, the tumor and metastases which initially had been palpable in the abdominal region had completely disappeared.

Conversely, in rabbits in which the initial lability of the nerve cells of the hypothalamus and the sympathetic ganglia was not sufficiently high, the increased excitation of the cerebral cortex during growth of the tumor induced reflexly a state of lowest activity in these cells, leading to the development of inhibition in these structures. Since it developed in the first place in the sympathetic ganglia, it rendered impossible the regulation of the trophic processes in the tissues of the central nervous system. With cessation of the flow of impulses in the peripheral part of the sympathetic nervous system, it is evident that the necessary increase in excitation of the cells of the nerve centers themselves, and in particular of the cerebral cortex, was absent, whereas their increased activity was essential to balance the trophic disturbances in the tissues arising under the influence of the developing tumor and mestastases.

In order to verify the importance of the higher functional activity of the autonomic nerve centers to the arresting of growth of a malignant tumor in the body, we carried out the following series of experiments. Chronic experiments were conducted on 10 rabbits into the cerebral cortex and hypothalamus of which electrodes had been introduced. Starting on the 15th-20th day after transplantation of the tumor (when the development of inhibition in the central nervous system under the influence of afferent impulses could be judged by the character of the bioelectrical activity of the cerebral cortex and hypothalamus; Fig. 3, 3) we gave intravenous injections twice a day for 40-45 days (1ml/kg body weight) of a drug combination of our own composition. This combination included drugs stimulating oxidation-reduction processes in the tissues and improving the synaptic transmission of impulses (riboflavin, thiamin, ascorbic acid, nicotinic acid, calcium chloride).

It was shown by daily observations of the bioelectrical activity of the nerve centers that, in those cases when the action of the drug combination brought about an improvement in the synaptic transmission of nerve impulses and in the functional state of the nerve cells, the inhibition developing in the synapses of the nervous system was abolished. This was observed in 6 of the 10 experimental rabbits.

After the action of the drug combination had begun, high bioelectrical activity was most rapidly restored in the sympathetic ganglia (which we checked by an acute experiment) and in the hypothalamus, not being directly under the action of afferent impulses (see Fig. 2b, 3, 4; Fig. 3, 4). Immediately after the increase in the activity of the autonomic nervous system, a persistent increase in the bioelectrical activity of the cerebral cortex also was observed, as shown by an increased amplitude of the slow and quick waves of the electroencephalogram (see Fig. 3, 4, 5).

In addition to this, from one to two weeks after the injections of the drug combination had started, cessation of growth and gradual reduction in the size of the tumor was observed, and 60 days after its transplantation into experimental animals, the tumor completely disappeared. In the last 8-10 days, absorption of the tumor took place despite the fact that injections of the drug were discontinued.

This was undoubtedly connected with the fact that the level of activity of the nerve centers continued to be raised. As the experimental results showed, against the background of the increased functional activity of the nerve centers and of the improvement in neuro-humoral regulation as a result of the action of the drugs composing the drug combination, an essential reorganization of the cells and tissues took place.

The results obtained from animal experiments in which the bioelectrical activity of different divisions of the nervous system was recorded against a background of the development of a transplanted Brown-Pearce tumor showed that the intensity of development of the tumor in the animal body was directly dependent on the functional state of the autonomic nervous system, and in particular, of the sympathetic nervous system. The sooner after the excitation(induced reflexly in the sympathetic nervous system by the flow of impulses to the center from the site of development of the tumor) that inhibition developed in the ganglia of the sympathetic nervous system, the more intensively did the tumor develop and produce extensive metastases, leading to the death of the animal. Conversely, the more resistant the centers of the hypothalamic region and the ganglia of the sympathetic nervous system (as a result of high lability of their cells) to the action of the stimuli reaching them reflexly from the periphery, the slower the tumor developed.

By maintaining the functional activity of the centers of the sympathetic nervous system with drugs stimulating oxidation-reduction processes, it was possible to create favorable conditions for the restorations of the disturbed synaptic transmission of nervous impulses through the ganglia.

This, in its turn, by enabling the regulation of the trophism of the tissues of the central nervous system, brought about a cytological reorganization of the tissues, leading to absorption of the malignant cells of the transplanted Brown-Pearce tumor.

SUMMARY

The bioelectrical activity of the cerebral cortex, hypothalamus, cerebellum and the superior cervical sympathetic ganglia was simultaneously recorded in rabbits against the background of a gradual development of a transplanted Brown-Pearce tumor.

The data obtained demonstrated that the intensity of tumor development depended on the functional condition of the autonomic nervous system, primarily of the sympathetic division.

The more rapid the development of inhibition reflex in the ganglia of the sympathetic nervous system and hypothalamus (following reflex excitation under the effect of impulses passing from the site of tumor development to the central nervous system) – the more accelerated the tumor growth with spread of metastases, this finally resulting in the animals' death. Conversely, the more stable (from high lability of the cells) the ganglia of the sympathetic nervous system to the action of reflex impulses from the periphery – the slower the development of the tumor. By raising the functional condition of the sympathetic nervous system with the aid of a number of substances stimulating oxidation-reduction processes, it became possible to maintain the excitation in the central nervous system for a prolonged period of time. In these conditions resolution of the transplanted Brown-Pearce tumor took place.

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THE RELATIONSHIP BETWEEN THE MOTOR CHRONAXIE OF ANTAGONISTIC MUSCLE GROUPS AND THE SUBORDINATIVE INFLUENCES OF THE HIGHER NERVE CENTERS IN THE PERIOD OF GROWTH OF A MALIGNANT TUMOR

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According to Bourguignon's law, the ratio between the chronaxie of the flexors and extensors is usually equal to two, or is very close to that value. This ratio between the chronaxie values may be called the subordinative index. As we have previously shown [1], in rabbits with a growing Brown-Pearce tumor, its absolute value is not only increased, but also distorted. Our observations are in agreement with the findings of several workers [1-10].

The problem of the link in the nervous system which exerts a definite effect on the quantitative aspect of these functional changes has not been solved. L. V. Latmanizova and her co-workers [3-7] sought to solve this problem by recording the action potentials of the cerebral cortex and the subcortical structures and comparing the results obtained with those of recordings of the functional state of the peripheral nervous system as studied by the methods of chronaximetry and accommodation tests. No convincing results were obtained, however [5].

In order to solve this problem we chose the method of successive exclusion, by means of appropriate measures, of the various links of the reflex arc, at the same time making observations on the chronaxie values.

EXPERIMENTAL METHOD

We divided 45 male chinchilla rabbits (weighing 2500-3000 g) into 4 groups: 10 rabbits in each of the 1st, 2nd and 3rd groups and 15 rabbits in the 4th group. The initial values of the chronaxie in each group were determined twice – before operation, and after it but before implantation of a tumor, which was performed in the right testicle of the rabbit as soon as the acute sequelae of the operation had cleared up. Further tests of the chronaxie were made during the time of growth of the tumor as follows: on the 1st, 5th, 10th, 15th and 20th days from the moment of transplantation. The chronaxie was determined by stimulation of the motor points of antagonistic muscle groups in the hind limbs, innervated by the sciatic and femoral nerves.

The individual links of the reflex arc were excluded surgically. The rabbits of the 1st group were subjected to denervation of the right testicle (the nerve fibers in the region of the spermatic cord were divided); completeness of denervation of the testicle was judged by absence of response to stimulation by the electric current. In the animals of the 2nd group, the sympathetic supply was interrupted in the thoracolumbar division, to the left of the spine (the sympathetic chain was divided in the thoracolumbar division on the left and the adventitia was removed from the wall of the aorta for a distance of 0.5 and 1cm in its abdominal portion). In the rabbits of the 3rd group the cerebral cortex was extirpated. The radical nature of the surgical procedures was verified at the end of the experiment by pathologoanatomical and histological examination. In the rabbits of the 4th group, unilateral division of the spinal cord was carried out at the level of the 10th thoracic vertebra (on the right side).

TABLE 1

Results of Chronaximetry and Values of the Subordinative Index in a Group of Rabbits after Denervation of the Right Testicle and Implantation of Brown-Pearce Carcinoma Cells

Rabbit no.	Initial background values					Values on the 30th day after operation					Values on the 15th day after implantation of the tumor				
	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)
	rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie	
490	5,50	0,26	11,75	0,13	+2,0	7,50	0,18	8,50	0,31	—1,7	6,62	0,36	11,75	0,15	+2,4
454	7,50	0,31	9,0	0,15	+2,1	3,50	0,21	10,50	0,16	+1,3	11,50	0,52	16,75	0,03	+17,3
542	4,75	0,41	8,25	0,21	+1,9	4,50	0,36	12,0	0,18	+2,0	6,75	1,09	35,50	0,21	+5,2
636	9,75	0,23	11,25	0,11	+2,1	4,25	0,41	8,25	0,16	+2,5	3,25	4,0	22,50	0,40	+10,0
547	6,25	0,22	12,0	0,12	+1,8	5,0	0,49	9,25	0,21	+2,3	8,80	0,26	17,87	0,26	+1,0
699	7,75	0,24	10,0	0,12	+2,0	7,50	0,21	16,25	0,10	+2,1	6,75	0,37	16,12	0,11	+3,3
495	5,50	0,26	11,75	0,13	+2,0	5,50	0,21	10,0	0,10	+2,1	4,37	1,24	20,28	0,19	6,5
111	7,25	0,26	14,0	0,13	+2,0	8,25	0,34	11,25	0,17	+2,0	10,37	0,82	17,50	0,29	+2,8
30	7,0	0,30	12,75	0,15	+2,0	6,25	0,42	10,0	0,21	+2,0	9,12	0,23	6,75	1,29	—5,6
695	4,75	0,22	14,25	0,11	+2,0	5,75	0,21	7,50	0,16	+1,3	Rabbit died				

TABLE 2

Results of Chronaxinetry and Values of the Subordinative Index in a Group of Rabbits after Sympathectomy and Implantation of Brown-Pearce Carcinoma Cells

Rabbit no.	Initial background values					Values on the 30th day after operation					Values on the 15th day after implantation of the tumor				
	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)
	rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie	
263	4,25	0,31	13,0	0,18	+1,7	12,25	0,29	18,75	0,15	+1,9	3,75	0,88	8,75	0,34	+2,6
290	7,75	0,31	9,0	0,15	+2,0	5,75	0,26	11,25	0,13	+2,0	5,0	0,26	12,50	0,08	+3,3
281	7,25	0,26	10,0	0,13	+2,0	5,75	0,41	20,0	0,19	+2,2	4,25	0,83	8,50	0,07	+11,9
283	5,50	0,26	11,75	0,13	+2,0	6,25	0,34	16,25	0,17	+2,0	3,25	0,47	19,50	0,07	+6,7
273	5,75	0,15	6,25	0,29	−1,9	10,0	0,21	12,0	0,10	+2,1	3,25	0,47	13,75	0,21	+2,2
75	9,25	0,21	16,25	0,11	+1,9	12,50	0,44	15,50	0,21	+2,1	7,50	0,78	13,25	0,08	+9,7
671	12,50	0,31	17,75	0,14	+2,2	9,0	0,21	14,75	0,31	−1,9	5,25	0,36	16,25	0,08	+4,5
685	6,75	0,25	12,50	0,11	+2,2	6,75	0,39	13,25	0,18	+2,2	5,0	0,67	14,25	0,28	+2,4
672	7,0	0,24	11,25	0,12	+2,0	8,25	0,47	17,50	0,22	+2,1	5,50	0,47	10,0	0,26	+1,8
673	6,50	0,19	10,75	0,10	+1,9	5,50	0,52	12,25	0,24	+2,3	3,75	0,41	13,0	0,13	+3,2

TABLE 3

Results of Chronaximetry and Values of the Subordinative Index in a Group of Rabbits after Extirpation of the Cerebral Cortex and Implantation of Brown-Pearce Carcinoma Cells

Rabbit no.	Initial background values					Values after operation					Values on the 15th day after implantation of the tumor				
	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)
	rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie	
264	12,50	0,31	17,85	0,14	+2,2	10,0	0,15	10,0	0,15	1,0	5,0	0,41	7,50	0,15	+2,7
285	9,10	0,26	18,0	0,13	+2,0	12,25	0,23	9,75	0,23	1,0	Rabbit died				
271	9,0	0,36	9,0	0,18	+2,0	5,50	0,52	5,50	0,39	+1,3	7,0	0,44	12,50	0,09	+4,9
257	10,25	0,22	15,0	0,11	+2,0	7,50	0,52	10,0	0,36	+1,5	5,0	0,47	10,0	0,47	+1,0
295	7,50	0,26	19,25	0,10	+2,6	9,50	0,15	9,50	0,15	1,0	4,75	0,36	8,75	0,09	—1,2
282	5,75	0,34	20,0	0,13	+2,6	12,0	0,52	10,75	0,15	+3,5	5,25	0,23	6,75	0,23	1,0
280	11,75	0,16	17,50	0,08	+2,0	6,0	0,23	6,25	0,23	1,0	4,50	0,47	10,50	0,07	+6,7
286	9,45	0,21	12,50	0,12	+1,8	8,75	0,31	8,75	0,31	1,0	3,75	0,41	8,75	0,13	+3,1
287	6,50	0,31	13,0	0,18	+1,8	3,75	0,36	15,75	0,10	+3,6	6,25	0,15	6,25	0,23	—1,5
200	7,50	0,24	10,0	0,13	+1,8	9,50	0,39	9,50	0,39	1,0	3,0	0,52	19,75	0,07	+7,4

TABLE 4

Results of Chronaximetry and Values of the Subordinative Index in a Group of Rabbits after Unilateral Division of the Spinal Cord at the Level of the 10th Thoracic Vertebra and Implantation of Brown-Pearce Carcinoma Cells

Rabbit no.	Initial background values					Values on the 10th day after operation					Values on the 15th day after implantation of the tumor				
	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)	sciatic nerve		femoral nerve		ratio between chronaxie values (subordinative index)
	rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie		rheobase	chronaxie	rheobase	chronaxie	
516	5,25	0,26	10,25	0,13	+2,0	7,50	0,26	9,50	0,31	—1,2	11,25	0,26	18,75	0,21	+1,2
517	4,75	0,28	7,75	0,12	+2,3	8,0	0,26	10,75	0,41	—1,6	5,75	0,26	15,0	0,26	1,0
518	6,50	0,22	14,25	0,11	+2,0	8,75	0,23	12,25	0,23	1,0	6,75	0,36	10,75	0,36	1,0
520	4,75	0,28	11,25	0,14	+2,0	10,0	0,52	10,25	0,39	+1,3	10,0	0,29	10,0	0,29	1,0
512	6,25	0,25	16,25	0,11	+2,2	11,25	0,18	10,0	0,18	1,0	2,75	0,47	5,0	0,47	1,0
622	3,50	0,28	11,25	0,12	+2,3	8,50	0,26	11,0	0,26	1,0	11,25	0,15	11,25	0,15	1,0
652	13,75	0,27	18,12	0,12	+2,2	9,50	0,13	11,75	0,13	1,0	6,0	0,23	14,50	0,15	+1,5
682	9,50	0,15	6,25	0,29	—1,9	7,25	0,25	10,75	0,34	—1,4	9,25	0,21	9,50	0,21	1,0

EXPERIMENTAL RESULTS

The observations showed that in the rabbits of the 1st group, in which the right testicle was denervated and a suspension of tumor cells was subsequently inoculated, the values of the chronaxie and of the subordinative index (Table 1) underwent the same quantitative changes as in the rabbits in which growth of a malignant tumor was taking place but which had been subjected to no form of operation on the nervous system. The same thing was observed in the animals of the 2nd group, after interruption of the sympathetic supply in the region of the thoracolumbar division and subsequent implantation of tumor cells in the right testicle (Table 2).

In the rabbits of the 3rd group, in which the cerebral cortex was extirpated, the chronaxie values were considerably altered (Table 3).

These changes took the form of partial equalization of the values of the chronaxies of the flexors and extensors in the postoperative period. In 2 of the 10 rabbits the subordinative index became equal to unity.

In the majority of animals, however, the values of the chronaxie and of the subordinative index were little different in the period after implantation of the tumor from the corresponding values in rabbits in which growth of a malignant tumor was taking place without extirpation of the cerebral cortex.

In the rabbits of the 4th group, in which the spinal cord was divided unilaterally at the level of the 10th thoracic vertebra, the changes in the chronaxie and subordinative index found in the rabbits of all the remaining groups in the period of growth of the malignant tumor were completely absent. The values of the chronaxie of the flexors and extensors of the hind limbs became almost identical, and remained constant during the experiment from the moment of operation (Table4).

The chronaxie changes which we observed in rabbits during growth of a malignant tumor, although they depended on the functional state of the peripheral links of the reflex arc and on the integrity and functional activity of the cerebral cortex, thus depended to no less degree on the influence of the divisions of the central nervous system lying between the cortex and the segment of the spinal cord at the level of the 10th thoracic vertebra.

SUMMARY

The purpose of this research was to ascertain which portion of the nervous system affects the relationship between the flexor and extensor chronaxie in the hind limb of rabbits after implantation of a Brown-Pearce carcinoma. Denervation of the testicle, thoracolumbar sympathectomy, hemisection of the spinal cord, and extirpation of the cerebral cortex were undertaken in groups of experimental animals. Changes in the chronaxie relationships were found in the last two groups but not in the first two.

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THE ACTION OF TUMOR ANTISERUM ON THE MITOTIC ACTIVITY OF AN EHRLICH'S ADENOCARCINOMA DURING PHYSIOLOGICAL EFFORT BY THE ANIMAL

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It has previously been shown [1, 4-6] that tumor antiserum depresses mitotic activity of the tumor cells of Ehrlich's adenocarcinoma, but has no essential effect on the intensity of division of the cells of the corneal epithelium [6] nor of the intestinal crypts [1]. It must be mentioned that in these experiments the injections of serum were given when the animals were in a normal condition and were being kept in the usual way.

It is also known that the mitotic activity of a tumor is altered when various factors act on the body [2, 3]. Since work by Bullough [12] has shown that the mitotic activity of the epidermis of normal mice is diminished by an obligatory increase in their motor activity, we thought it of interest to ascertain whether such a relationship between the physiological state of the animal and the mitotic activity was also typical of a tumor, and also to determine the effect of tumor antiserum on the intensity of division of tumor and normal tissue cells, when administered during motor activity by the animals.

In the present work we accordingly studied the relationship between the mitotic activity of a subcutaneous Ehrlich's adenocarcinoma and the enforced performance of increased motor activity by the animals, and the effect of tumor antiserum on the mitotic activity of this tumor when given under identical experimental conditions. In the second part of the investigation we studied the intensity of division of the corneal epithelium and the intestinal crypts, as well as the tumor cells in the same experimental animals.

EXPERIMENTAL METHOD

In the first experiment we used 28 male white mice. The experiment took place during the morning (from 10:45 A.M) of the 9th day after subcutaneous transplantation of the tumor. Twenty mice were placed in a smooth-bottomed box, fixed to an apparatus which produced a uniform to-and-fro movement, to which the mice were subjected for 30 minutes; as a result they were kept in a state of increased motor activity during this time. Two groups of control animals (with 4 mice in each) were kept under ordinary conditions.

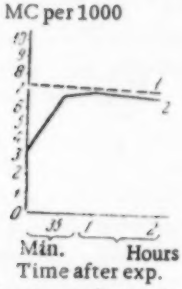
In order to determine the trend of the changes in mitotic activity of the tumor after cessation of the increased motor activity, the 20 experimental mice were divided into 4 groups with 5 mice in each. Each group of animals was killed at different times: group I — as soon as the experiment ended, group II — after 35 minutes, group III — after 1 hour and group IV — after 2 hours. The first control group of mice was killed at the same time as the first experimental group, and the second control group at the same time as the fourth experimental group. The tumors were fixed in Zenker's fluid, stained by Feulgen's method, and counterstained with light green.

The second experiment was carried out on 40 male white mice, which were divided into 4 groups, each of 10 animals. An Ehrlich's adenocarcinoma was implanted subcutaneously in the dorsal region of all the ani-

Mitotic Coefficient in a Subcutaneous Ehrlich's Adenocarcinoma after Injection of Tumor Antiserum

Mouse no.	Serum + motor activity (group I)	Serum under ordinary conditions (group II)	Motor activity alone (group III)	No additional agent (group IV)
1	2.26	3.82	6.15	6.74
2	5.52	4.40	6.33	7.26
3	5.94	4.57	6.45	7.32
4	6.18	4.70	7.39	7.40
5	6.24	5.01	7.49	8.00
6	6.30	5.37	9.24	8.57
7	7.84	5.52	9.77	8.60
8	7.98	5.78	10.16	8.98
9		6.20	12.69	10.62
10	Mouse died	6.39		17.24
Mean MC	6.03	5.18	8.14	9.07

mals. The mice of group I and III were kept in a state of increased motor activity for 30 minutes in the morning and evening for 4 days, starting on the 7th day after implantation of the tumor. Just before the beginning of the experiment in the evening (at 4 P.M.) on the 8th, 9th and 10th day of development of the tumor, the group I mice received an injection of 0.5 ml of serum beneath the skin of the dorsal region at a short distance from the tumor. The group II mice, kept under the usual conditions, received injections of serum by the same method and at the same times on the same days, but the group IV animals received no extra treatment in any form.



Mitotic coeficient in Ehrlich's adenocarcinoma after the cessation of increased motor activity by the animals. 1) control; 2) experiment.

The sera which we injected into the mice of groups I and II were obtained by intravenous immunization of chinchilla rabbits and tested by the complement fixation reaction at 37°. The sera reacted with tumor antigen in a dilution of 1:400 +++, and with antigens from liver and spleen in lower dilutions (1:200 ++ or 1:100 +++).

The experimental animals of all groups in the second experiment were sacrificed at 11 hours on the morning of the day following the last injection of serum. It was useless to sacrifice them sooner after the injection of serum since, according to the findings of P. I. Tseitlin and M. M. Kapichnikov [10], tumor antiserum increases the intensity of renewal of the protein tumors for roughly 20 hours after injection.

The tumors and intestine were fixed in Carnoy's fluid, and the cornea in a 5% solution of glacial acetic acid in 70° alcohol. All the material was stained by Carazzi's hematoxylin.

The mitoses in the mouse tumors in the first experiment were in each case counted in 500 fields of vision (about 100 000 cells from each tumor were examined). In the second experiment in the number of mitoses in the tumor was counted in 300 fields of vision (roughly 70 000 cells were examined), and the corneas — on both sides of two mutually perpendicular lines drawn in ink on the cover glass of the preparation (approximately 35 000 cells), and in the intestine — in 50 crypts, cut longitudinally (about 3500 cells). After counting the mitoses, we calculated the mitotic coefficient MC (the ratio between the dividing cells and the total number of cells in this preparation examined, per 1000) and the coefficient K (the ratio between the sums of the early and late phases of division). The experimental results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

First experiment. Counting the mitoses in the tumors in the mice of the first experiment showed that the mean percentage of the various phases of division in the experimental and control groups varied between narrow limits (P ≈ 4-7%; M ≈ 77-83%; A ≈ 5-8 % and T ≈ 7-11%), just as did the coefficient K (from 5 to 7).

The changes in the value of MC at different times after the end of the experiment are shown in the form of curves on the figure.

We see that the value of MC in the Ehrlich's adenocarcinoma was least (3.22 per 1000) in the experimental mice sacrificed immediately after cessation of increased motor activity; 35 minutes later the value of MC increased sharply to 6.92 per 1000 (the difference between the MC of this group and of the preceding group is significant $p = 0.004$). At later periods of investigation (after one and two hours) the value of MC remained at roughly the same level (7.03 per 1000 and 6.95 per 1000).

In the first control group of mice MC was equal to 7.31 per 1000, and in the second, 7.20 per 1000. The difference between the MC of mice sacrificed immediately after the end of the experiment (3.22 per 1000) and the MC of the mice of the first control group (7.31 per 1000), sacrificed at the same time as this experimental group, was statistically ($p = 0.015$). The mitotic activity in the subcutaneous Ehrlich's adenocarcinoma thus fell sharply after the end of the increased motor activity by the animals and was restored to the control level 35 minutes later.

The experimental results were in agreement with the findings of Bullough [12], obtained for normal tissue cells of animals exposed to similar conditions, and they suggest that the mitotic activity of tumor cells is to some extent dependent on the physiological state of the animal, which is also characteristic of the cells of normally derived tissues [7-9, 11].

Second experiment. During the period of the experiment one mouse from group I died from other causes; in a second animal the mitotic activity of the tumor cells could not be examined on account of its small dimensions (that of a grain of wheat). In one mouse of group III the mitotic activity of the tumor cells could not be investigated because of its extensive necrosis.

Counting the mitoses in the tumors of the remaining mice showed that the mean percentage of the various phases of division varied in all the groups studied between narrow limits (P ≈ 3-6%; M ≈ 75-80%; A ≈ 3-4%; T ≈ 12-16%), just as did the coefficient K (from 4 to 5%). The values of the mitotic coefficient for the tumors of the experimental animals of all the groups are given in the table.

It can be seen from the figures given in the table that the smallest, and roughly equal, mean values of MC were observed in the tumors of the group I animals, in which serum was injected in conjunction with motor activity (MC = 6.03 per 1000), and in those of the second group, in which serum was given under ordinary conditions (MC = 5.18 per 1000). In the mouse tumors of groups III and IV (no serum injected), the MC was considerably higher and also roughly equal (group III – 8.41 per 1000, group IV – 9.07 per 1000). The difference between MC for groups I and III and for groups I and IV was significant ($p = 0.024$), as also was that between groups II and III and between groups II and IV (in the first case $p = 0.000$ and in the second, $p = 0.002$).

As a result of the counting of the mitoses in the corneal epithelium of the experimental mice it was found that no essential differences were present in the proportions of the phases of division between the groups (P ≈ 15-28%; M ≈ 33-46%; A ≈ 6-8%; T ≈ 22-37%), just as in the case of the value of the coefficient K (≈ 2). The mean values of the MC in the corneal epithelium of the mice of all four groups varied within narrow limits (group I-4.02 per 1000, II-3.57 per 1000, III-3.19 per 1000 and IV – 3.16 per 1000); the difference between them was not significant.

Counting the mitoses in the crypts of the small intestine of the mice showed that the mean percentage of the different phases of division in all four groups differed only slightly (P 6-9%; M 53-60%; A 4-6%; T 26-35%), just as did the ratio between the early and late phases of division (K ≈ 2). The mean values of the MC differed slightly in the various groups (group I – 28.82 per 1000, II – 34.20 per 1000; III – 32.85 per 1000 and IV – 31.67 per 1000), but the difference between the MC of all the groups was not significant.

The results of the second experiment thus showed that the mitotic activity in the tumors of mice sacrificed on the day following increased motor activity was unchanged from that in control animals kept under ordinary conditions, as might have been expected from the results of the first experiment.

From an analysis of the results of the second experiment it may also be concluded that tumor antiserum, when injected for a period of 3 days into animals kept under unaccustomed conditions (periodic enforced increased motor activity), possesses the same depressing action on the intensity of division of the cells of an Ehrlich's adenocarcinoma as when injected in ordinary conditions. At the same time, when tumor antiserum is injected

into mice in which an Ehrlich's adenocarcinoma is developing subcutaneously, kept under ordinary conditions or on repeated physiological effort (increased motor activity), it caused no essential changes in the corneal epithelium nor the crypts of the intestine of the experimental animals.

SUMMARY

As demonstrated on 68 white mice the mitotic activity of Ehrlich's tumor becomes temporarily reduced directly after intense motor activity by the animals. Anti-tumor serum administered to mice kept in the ordinary way or engaged in intensive motor activity depressed the mitotic activity of the tumor cells but did not exercise any significant effect on the intensity of cellular division of the corneal epithelium nor on that of the intestinal crypts in the same experimental animals.

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EXPERIMENTAL BIOLOGY

THE ALKALINE PHOSPHATASE ACTIVITY OF THE TISSUES AND THE PRIMORDIAL GERM CELLS DURING THE EMBRYONIC DEVELOPMENT OF THE MOUSE

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Among the large volume of research devoted to the study of the alkaline phosphatase activity in the cells of mammals, a specific place is occupied by histochemical investigations of this enzyme in those cells of the embryo which are usually called the "primordial germ cells". In these reports [4, 5] and also in certain textbooks [3], it emphasized that high alkaline phosphatase activity is a specific sign of the primordial germ cells, distinguishing them clearly from the remaining tissues of the embryo.

This point of view, of importance in principle, is based, however, on a limited amount of factual evidence, comprising only isolated stages of embryonic development. The alkaline phosphatase activity of the developing reproductive glands of the embryo, and also at early stages of embryogenesis, has not been studied.

We accordingly set out to investigate the alkaline phosphatase content throughout the entire period of intrauterine development of the mouse, starting with the ovum.

EXPERIMENTAL METHOD

The age of the embryos was determined from the moment of impregnation of the female; the interval between each two successive stages of development was one day.

The alkaline phosphatase was detected by Gomori's histochemical method, as modified by V. V. Portugalov [1]. In order to minimize inactivation of the enzyme, the treatment of the material from fixation to incubation in the substrate inclusively lasted no more than 24 hours.

The optimal duration of incubation, which was determined empirically, was 1 hour, but in the case of certain sections of the embryo this period was deliberately lengthened to 16 hours and also shortened to 15-30 minutes.

In each experiment, starting from the blastocyst stage, only half the total number of embryos found in the uterus was used for determination of the alkaline phosphatase, the rest being fixed in Zenker's fluid and, after the usual histological treatment, stained by Feulgen's method for DNA and also with hematoxylin-eosin. This enabled the alkaline phosphatase activity to be compared with certain other properties of the primordial germ cells.

In all 109 embryos were investigated, 53 by Gomori's method. In order to judge the degree of alkaline phosphatase activity, in the experiment consideration was paid in the first place to the intensity of staining of the cell by Gomori's method, using the conventional nomenclature of I. I. Sharov [2], and in the second place to the shortest duration of incubation necessary to show the enzymic action of the phosphatase. As a rule the two criteria agreed with each other.

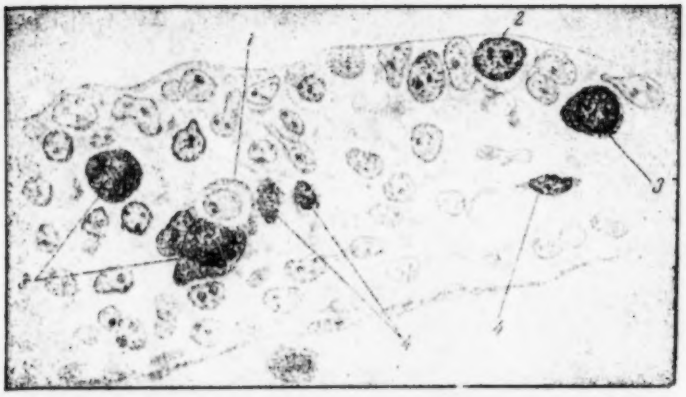

Fig. 1. Area of the germ layer of a 9-day mouse embryo. Stained by Gomori's method. The primordial germ cells (1, 2, 3) show transition from feebly (1) to intensively (3) stained. The individual nuclei of the symplast (4) are also well stained. (The entire figure was produced by means of the Abbe drawing apparatus at the level of the objective table of the microscope under a magnification of 7 × 9).

Fig. 2. An area of the undifferentiated sex glands of a 12-day mouse embryo. Alongside the primordial germ cells (1), staining well by Gomori's method, there are others (2) almost unstained. Some nuclei of the symplast are well stained (3).

EXPERIMENTAL RESULTS

The unfertilized oocyte, the polar bodies, zygote, blastomeres and all the cells of the blastocyst contain only traces of alkaline phosphatase, forming a sharp contrast to the tissues of the oviduct or uterus surrounding the embryo. Even after increasing the exposure to 3-4 hours, only the membranes of the cells and nuclei and also the nucleoli were stained.

In the neurula stage (5 days after fertilization) differences in the phosphatase activity were first seen: the neural tissue contained more alkaline phosphatase than the rest of the body of the embryo.

In embryos aged 6-7 days alkaline phosphatase was found in the neural tissue and in the entoderm of the hind gut. In contrast to the neural tissue, the cells of the entoderm were stained unevenly by Gomori's method; transitional stages were seen from cells stained as darkly as the neural cells to others quite lightly stained. The entodermal cells which possessed differing phosphatase activity were in general similar to each other in their dimensions and in the sharp and size of their nucleus, and also in its internal structure and DNA content. The intensity of staining by Gomori's method, even in the darkest of these cells, was much weaker than in the primordial germ cells appearing in the course of the further development of the embryo.

In 8-9 day embryos many organs could be clearly distinguished, but the germ layer was not yet visible. Alkaline phosphatase was present in considerable quantity in all the cells of the spinal cord, in the tissue lining the vesicles of the brain; and in the entoderm of the foregut. A particularly high phosphatase activity, not previously found was shown by individual cells situated mainly in the tissue of the hind gut (ento- and mesoderm), and also in the wall of the abdominal aorta and the umbilical vein (solitary cells). The number of such cells in a section was 1-3. They were arranged singly and haphazardly and some were undergoing division by mitosis; under these circumstances the chromosomes were stained even more intensively than the cytoplasm by Gomori's method.

The tissue of the wall of the gut surrounding the cells, poor in alkaline phosphatase is in the form of a

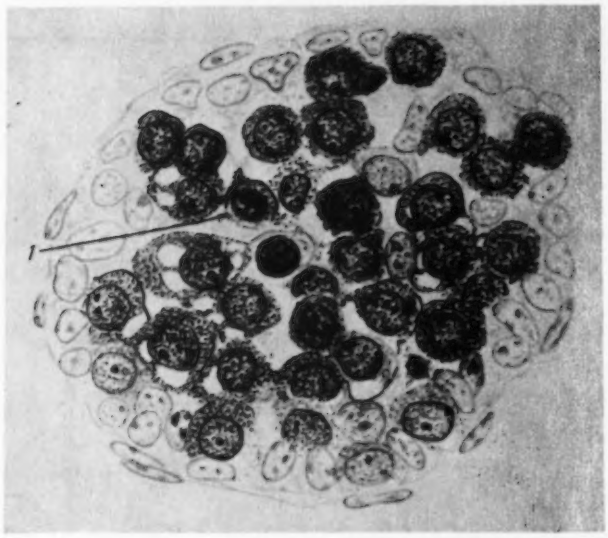

Fig. 3. Transverse section through a testicular cord of a 16-day mouse embryo. Nearly all the primordial germ cells stained intensively by Gomori's method, in contrast to the majority of nuclei of the symplast. 1) degenerating primordial germ cell.

symplast. The structure and staining of the nuclei with hematoxylin and by Feulgen's method were very similar in the cells and the symplast. Some cells were indistinguishable from the symplast in their phosphatase activity, i.e., they contained only traces of the enzyme; between these and the cells staining intensively by Gomori's method there were gradual transitional forms. The individual nuclei of the symplast were also stained intensively, but the cytoplasm belonging to them remained light.

On the 10th day, in many mouse embryos a germinal layer developed on the ventromedial surface of the primordial kidney. When stained with hematoxylin or by Feulgen's or Gomori's method, the tissue of the germ layer and the adjoining mesentery had the same structure as did the wall of the gut at the preceding stage of development. It consisted of a symplast, staining weakly by Gomori's method, and a small number of cells rich in alkaline phosphatase, together with cells which, in their structure and phosphatase activity, appeared to be transitional forms between a symplast and cells (Fig. 1).

In the wall of the aorta, the gut, and the umbilical vein there were also solitary cells containing very much alkaline phosphatase. An average degree of phosphatase activity was observed in the spinal cord and brain and in the optic vesticles; weak activity in the entoderm of the gut. The detritus from dead cells in the lumen of the gut stained well by Gomori's method.

At the undifferentiated gonad stage (11-13th day of intrauterine life) and the first time after the differentiation of the sex (14-15th day) the number of primordial germ cells increased rapidly as the result of their intensive division.

Only a proportion of these cells and many nuclei of the symplast stained by Gomori's method. The intensity of staining of each varied within wide limits (Fig. 2). After prolonged incubation the entire parenchyma of the gland appeared uniformly dark.

Besides the primordial germ cells, therefore, the symplast of the sex gland also possessed a much higher phosphatase activity than the majority of tissues of the embryo. This phenomenon was not due to diffusion of products of cleavage, since the staining was strictly localized in character and did not extend to the tunica albuginea nor to the interstitial tissue of the testis, nor to the tissue of the primordial kidney in immediate contact with it. The germinal epithelium of the testis was darker than the tunica albuginea with any exposure, but lighter than the testis cords.

Around the aorta and the cardinal veins were arranged many irregularly round "islets", characterized by a very high concentration of alkaline phosphatase, even higher than that in the sex glands. Inside a large islet were 10-20 cells and nuclei of a symplast, the small islet consisting of only a few cells. Solitary cells were also encountered which stained darkly by Gomori's method. In ordinary histological preparations, the tissue surrounding the aorta and the cardinal veins contained a considerable number of cells and nuclei of a symplast, arranged singly and in groups and morphologically similar to those in the sex gland. They probably form islets rich in alkaline phosphatase.

Isolated islets were present in the mesentery and the wall of the gut. The majority of islets lay at the level of the gonads, but they were also found outside the limits of this zone in both cranial and caudal directions. This displacement of the islets, and also the presence of a large number of them after completion of sexual differentiation, prevented them from being looked upon as primordial germ cells migrating to the gonad.

At first the spinal cord and brain contained alkaline phosphatase, but its activity fell, and it was not found in all the cells but only in certain areas.

On the following days of intrauterine life (15th-18th days) essential changes took place in the primordial germ cells. Their DNA content was greatly reduced, so that their nuclei were hardly stained by Feulgen's method. They ceased completely to multiple by mitosis, they increased in size and their cytoplasm became vacuolated. Very many cells degenerated.

Nevertheless the alkaline phosphatase activity of the primordial germ cells continued to increase, and the overwhelming majority of them had a very high content of this enzyme (Fig. 3). The contents of the vacuoles did not stain by Gomori's method. The nuclei of the symplast, rich in DNA and multiplying vigorously, now showed (with solitary exceptions) only traces of alkaline phosphatase. No relationship was thus observed between the DNA content and the alkaline phosphatase activity. The degenerating primordial germ cells had the most intensely staining nuclei both by Feulgen's and by Gomori's method.

There was considerable but uneven phosphatase activity in the developing cartilaginous tissue, the perichondrium, and the wall of the ureter (except the mucous membrane). Only traces of phosphatase remained in the spinal cord.

In the last days of intrauterine life a large quantity of alkaline phosphatase appeared in the subcutaneous cellular tissue and in the developing skin glands, coinciding in the second case with an increased quantity of DNA.

There were not, therefore, sufficient grounds for regarding the presence of high alkaline phosphatase activity as a distinguishing feature of the primordial germ cells. Large amounts of this enzyme were also present in other cells of the embryo.

At early stages of the development of the mouse embryo, no cells rich in alkaline phosphatase were observed, and their subsequent formation coincided with the appearance of cells, intermediate in their phosphatase content, in the tissues around them.

SUMMARY

A comparative study of the alkaline phosphatase activity was made in various tissues and primordial germ cells of white mice embryos by using the histochemical method of Gomori, modified by V. V. Portugalov.

Contrary to the commonly accepted opinion it was established experimentally that high alkaline phosphatase activity is an important, but by no means a specific nor strictly constant, property of the primordial germ cells.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE NATURE OF VIRUS-LIKE INCLUSIONS IN HUMAN RED BLOOD CELLS

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A number of papers has recently been published [2-9], reporting the results of examination of the red cells of man and animals suffering from various diseases, by means of the electron microscope.

These authors report that in many cases they have succeeded in finding submicroscopic particles in patients' red cells which are not present in the red cells of healthy persons.

On the films shown by these authors it is possible to see particles, round, oblong or irregular in shape, with ill-defined edges. In some cases the dimensions of the particles varied but little, in others – very considerably (from 100 to 450 mμ in infectious mononucleosis and from 20 to 120 mμ in Hodgkin's disease). In the authors' opinion, these particles resemble viruses.

In view of these findings, we made a comparative study of the red cells of patients suffering from cancer and of healthy persons.

EXPERIMENTAL METHOD

Films were made in accordance with the technique described in a paper by Reagan and his co-workers [5].

To 2 drops of blood, taken from the finger, was added 2 drops of physiological saline containing heparin (10 mg heparin to 10 ml physiological saline). The mixture was kept for 1 hour in the refrigerator and for 10 minutes at room temperature with occasional agitation.

Drops of the mixture were placed on a collodion film and excess fluid drawn off with a pipette. The films were washed with 6-7 drops of distilled water and, after drying, were dusted with nichrome at an angle of 15°.

The total magnification (electron-optic + photographic) of the photographs shown is approximately 8000.

EXPERIMENTAL RESULTS

In spite of the fact that the films had been carefully washed with distilled water, in many red cells there were remnants of hemoglobin in the form of clumps or granules of various sizes.

In some cases the grains of hemoglobin were very reminiscent of the formations described by Reagan, for example, in infectious mononucleosis (it must be remembered that Reagan's illustrations here are negatives). Structures of this type could be seen in both the red cells of patients suffering from cancer (Fig. 1, a) and the red cells of healthy persons (Fig. 1, b).

During hemolysis of red cells with distilled water, defects of various sizes frequently develop in their mem-

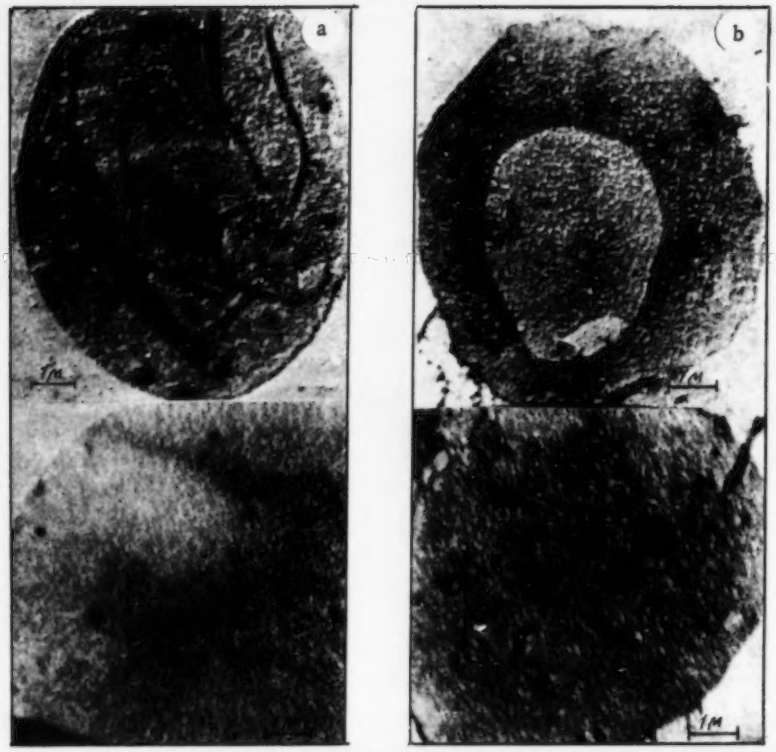

Fig. 1. Hemoglobin granules, capable of being mistaken for virus inclusions. a) red cells of patients suffering fron cancer; b) red cells of healthy persons. Magnification approximately 8000.

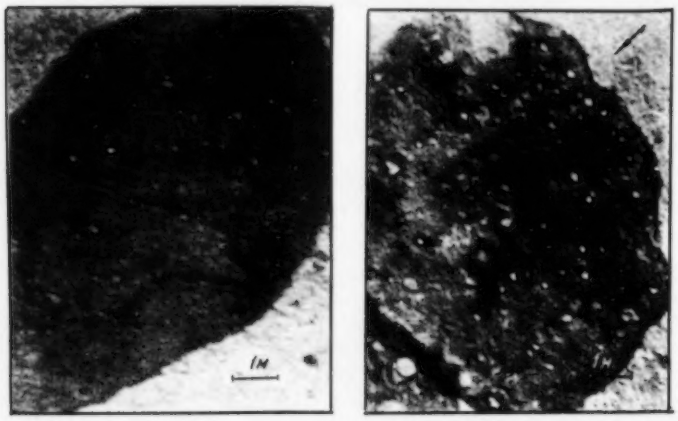

Fig. 2. Defect in the membrane, resembling submicroscopic particles. On the left – red cells of a cancer patient; on the right – red cells of a healthy person. Magnification approximately 8000.

branes (a large defect is seen in Fig. 1, b). Small depressions, after shading, resembled the particles described by Reagan in the red cells in Hodgkin's disease [5], and which had previously been observed by Frajola and his co-workers [1]. Numerous small defects in the membrane of the red cells are seen in Fig. 2, a (red cell from a cancer patient) and in Fig. 2, b (red cell of a normal person).

On the next film the defects are particularly reminiscent of small particles and only from the position of the shadows (the direction of shading is shown by the arrow) can it be seen that these are defects in the membrane.

SUMMARY

The author was unable to discover any difference in the erythrocytes of cancer patients and healthy persons by electron microscopic investigation.

Hemoglobin granules and slight defects similar to the formations revealed by Reagan in erythrocytes of patients suffering fron virus diseases were observed in the erythrocytic stroma of both healthy persons and cancer patients.

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• In Russian.

THE GROWTH OF CAPILLARIES IN THE BRAIN OF ADULT ANIMALS

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In the brain of man and of a number of animals (rats, rabbits, cats, dogs, etc.) the development of the vascular system continues for a long time after birth. Growth of capillaries by means of the outgrowth of buds, as described by B. N. Klosovskii [1] by his impregnation method, is an important process in increasing the general density of the capillary network and in forming the necessary relationships between the nerve cells and the capillaries in the postnatal period of development.

B. N. Klosovskii [2] considers tnqt the newly formed capillary is derived from an adventitial cell passing along a vessel. The process of its transformation into a functioning capillary includes several states. These stages of development of the capillaries in the brain were designated the stage of "budding" (the earliest stage), the stage of "polyp formation" and the "hydriod" stage (Fig. 1, a, b, c). Starting with the earliest stage, the growing capillary or outgrowth has well-marked protoplasmic processes, emerging from the free end of the capillary, where the nucleus of the adventitial cell is usually situated. As it grows, the bud reaches a functioning capillary, permeable to the blood stream, and joins itself to the capillary by means of the protoplasmic processes (see Fig. 1, d). After the union of the growing capillary bud to the functioning capillary, the protoplasmic processes disappear and the newly formed capillary begins to function.

The formations of new capillaries in this manner takes place in the brain not only in the embryonic period but also for a long time after birth. In puppies and kittens, for instance, growing capillaries are found at the age of 4-5 months, in rats at the age of 2 months [2-4]. Capillary growth is particularly intensive in the first days of postnatal life of the animal; with age this process gradually subsides.

The question of the possible growth of capillaries in the brain of adult animals has remained unanswered, for it has not been possible to find the characteristic forms of capillaries in process of construction in these animals.

When investigating the influence of various agents on capillary growth in the brain of animals in the postnatal period of development, we first studied in detail a complete series of sections of the brain of normal adult rats, prepared by B. N. Klosovskii's method. Examination of the silver-impregnated preparations revealed growing capillaries in the brain of certain of the rats.

EXPERIMENTAL METHOD

For investigation of the brain for the presence of growing capillaries we took 10 sexually mature rats weighing from 225 to 310 g (4 males) and from 140 to 185 g (6 females). All the rats were sacrificed by decapitation. The brain was fixed in 10% formalin. Using B. N. Klosovskii's method [2], we prepared series of sections

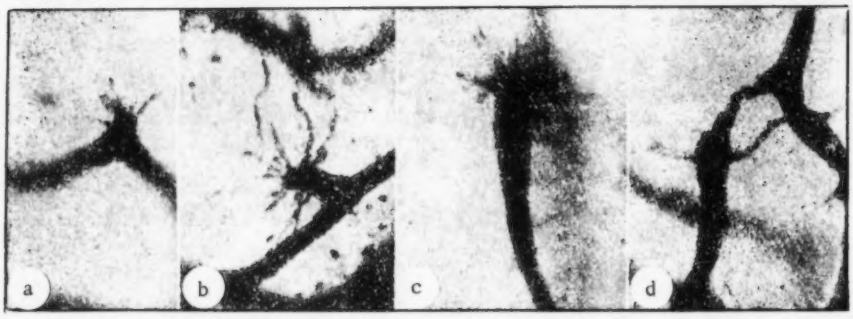

Fig. 1. Phases of growth of capillaries in the brain of normal 13-day old rats. a) "bud" stage; b) "polyp" stage; c) "hydriod" stage; d) union of the growing and functioning capillaries.

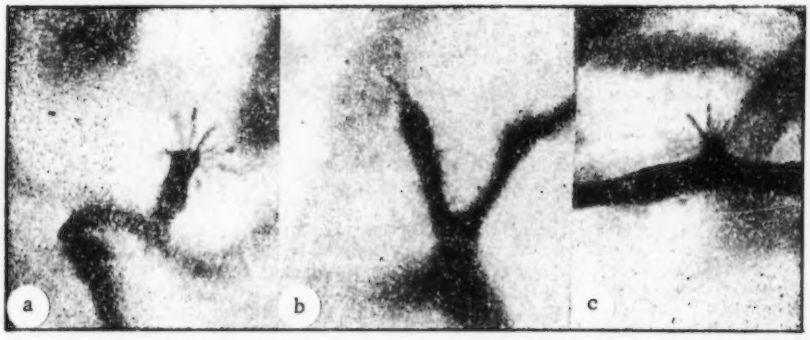

Fig. 2. Growing capillaries in the brain of normal adult rats. a, b) "hydriod stage"; c) "bud" stage.

through the whole brain to a thickness of 150 μ. The sections were examined under the microscope with a magnification 400x at all turns of the micrometer screw. This degree of magnification enabled the various forms of growing capillaries to be seen most clearly. Because of the small size of the rat's brain it was possible to examine the capillary network in all its divisions in a full series of sections.

EXPERIMENTAL RESULTS

In 6 of the 10 experiments we were able to find growing capillaries in various divisions of the brain (in 2 males and 4 females).

In contrast to developing animals, in which growing capillaries are found in large numbers in various divisions of the brain, in adult rats solitary growing capillaries were observed, and in only one part of the brain in each animal. For instance, growing capillaries were found in the olfactory gyrus at the level of the parietal region (one rat), in the frontal region — in the first layers of the cortex (2 rats), in the parietal region of the cortex (one rat), in the optic tubercle (one rat) and in the cerebellum and medulla oblongata (one rat).

The growing capillaries in the brain of the adult rats were mainly in the "hydriod" stage (Fig. 2, a, b), but capillaries in the "bud" stage were encountered (see Fig. 2, c). In all the growing capillaries there were well-marked protoplasmic processes, as may be seen from the microphotographs (see Fig. 2), and a single nucleus. The diameter of the pedicle of the growing capillary was the same as that of the surrounding functioning capillaries.

Besides capillaries in no way distinguishable from the ordinary normal growing capillaries in young animals,

some were seen which showed atypical growth: these either had no protoplasmic processes at all, or they appeared to be greatly curtailed. In some cases the pedicle of the growing capillary was very slender, and at its end were seen 1-2 diminutive protoplasmic processes.

The findings described thus show that capillary growth may take place in the brain of adult animals by means of the outgrowth of buds. However, in the brain of adult animals, the number of growing capillaries is limited, and their growth is localized to any one area of the brain in each animal, and does not extend to other areas.

No explanation has yet been found of what is responsible for the growth of the capillaries, of what factors — local or general in character — stimulate the formation of new capillaries during the normal existence of the animal. It is most probable that capillary growth in each individual case is due to local factors such as the need for adaptation to the changing demands of the nerve cells. There is also the possibility, as pointed out by B. N. Klosovskii [1, 2], of a reaction to the disintegration or death of individual nerve cells.

The fact that new capillaries may be formed in the brain of adult animals gives grounds for the belief that in certain conditions and in pathological cases the vascularization of individual areas of the brain may be increased by some means or other, should this become necessary. The action of different chemical and physical factors on the formation of new capillaries in the brain of animals of various ages will be the subject of our further research.

SUMMARY

To reveal newly formed capillaries the brain of normal adult rats was impregnated with silver by B. N. Klosovskii's method.

The possibility of formation of new capillaries in various portions of the brain of adult rats was established. In 6 of the 10 cases newly developing individual capillaries were revealed. By their external appearance these did not differ in any way from those developing in the nervous system of young animals.

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METHODS

THE IMPORTANCE OF THE INTERELECTRODE RESISTANCE DURING REGISTRATION OF BIOELECTRICAL PROCESSES FROM THE SKIN SURFACE IN MAN

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In a previous paper [1] we reported a method of recording electrocardiograms and electromyograms of industrial workers by means of a comparatively simple apparatus and suitable technique.

In order to secure the required electrical contract between the electrodes and the skin of the subject, at the point where the electrodes are to be applied, the skin is preliminarily treated with alcohol or other substances, because the stratum corneum of the epidermis and the secretion of the sebaceous glands have a high resistance to the electric current. In order to remove part of the stratum corneum of the epidermis for registration of electrocardiograms, the skin is rubbed with an electrode paste containing abrasives, for example finely ground pumice. For the registration of electromyograms and electroencephalograms the skin is simple degreased by being rubbed with alcohol or a mixture of alcohol and ether.

It is considered that after treatment of the skin of the subject with alcohol, when the electrodes used have a diameter, for example, of 10 mm, the interelectrode resistance has a value of the order of 10 kiloohm. Input resistance of the amplifier is greater (1 meg), an interelectrode resistance of this magnitude causes no perceptible distortion of the recorded electrogram.

Experience has shown that these assumptions are not always justified. The measurement of the interelectrode resistance in electrographic practice is performed as the exception, and there is no standard method by which it may be done.

The present paper is devoted to the study of the role of the interelectrode (mainly skin) resistance during registration of the bioelectrical processes from the surface of the skin in man.

Our aims were as follows: to ascertain whether distirtions of amplitude and frequency take place, and in what cases, as a result of a high interelectrode resistance, dependent on the frequency; to find out how this resistance changes with time (in the course of 8 hours); to find an effective method of lowering the interelectrode resistance; to suggest a standard method of measurement of the resistance and to determine criteria of reliability of the skin treatment. Our investigations showed that during treatment of the skin of the forearm with alcohol, and with values of the measuring current below the threshold of sensation, for example 5–10 μa, the value of the interelectrode resistance reached 1 meg. If the value of the input resistance of the amplifier was, for example, 1 meg, with an interelectrode resistance of this value, a fall in the amplitude of the electrical activity registered might be expected, on account of the fall of part of the voltage at the interelectrode resistance (amplitude distortions).

The dependence of the magnitude of the interelectrode resistance on the frequency of the measuring current gave grounds for thinking that electrical activity would be recorded with obvious weakening of the slow

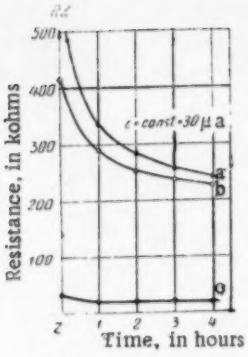

Fig. 1. Relationship between the value of the interelectrode resistance and the time elapsing after application of the electrodes to the subject's skin (electrogram taken with a current of 30 μa). Curves a, b and c were taken with electrodes applied to the forearm: a) and b) after treatment of the skin with alcohol; a) by the action of a direct current, b) by an alternating current with a frequency of 20 cps, c) by direct current, after combined preparation of the skin.

frequency components (fractions of and unit cps), and that the weakening of the higher frequencies would be less, the higher their frequency (frequency distortions).

These dangers were well-grounded, for the shape of the curves of electrocardiograms is known to be distorted when there is a high interelectrode resistance at the time of recording [7].

An effective lowering of the interelectrode resistance is essential in order to exclude amplitude and frequency distortions, to lower the magnitude of the induction voltage of the alternating current and, as we were able to show, to remove obstacles arising when the subject touched grounded objects (the stands, a grounded instrument and so on.

In order to solve these problems, we were unable to make use of any human electrical activity, for we had no power to change its amplitude and frequency. For this purpose we used a generator of low-frequency electrical sinusoidal oscillations with a measurement current below the threshold of sensation (less than 100 μa). We considered that if the laws of change of interelectrode resistance obtained with this generator were confirmed for any particular case of investigation of the interelectrode resistance by means of human electrical activity, it might then be considered that these laws were applicable in other cases.

We determined the magnitude of the interelectrode resistance to direct and alternating current, ny means of an ammeter-voltmeter scheme.

According to reports in the literature, the magnitude of the interelectrode resistance depends on the site of application of the electrodes on the subject's body [5], on the area of the electrodes, the quality of the skin treatment, the quality of the electrode paste or fluid [6], the temperature of the air around the subject [2], the type of current (direct, alternating, pulsed), the frequency of the current and its strength [3, 4] and the time taken by the current to pass through the interelectrode circuit.

In the first part of the investigation, we used ordinary skin preparation with alcohol.

In order to obtain comparable results during measurement of the interelectrode resistance, we used a standard direct current of 10 μa, and the measurement was carried out quickly (the resistance falls if the current passes for a long time).

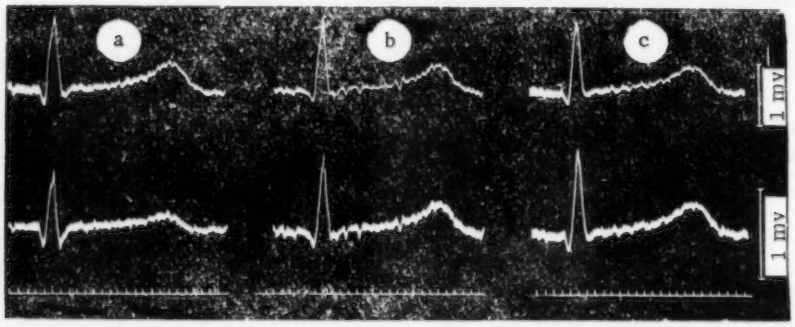

Fig. 2. Amplitude and frequency distortions after preparation of the skin of the subject's forearm with alcohol, and their change with time. For explanation, see text.

A relationship was found between the magnitude of the interelectrode resistance and the time elapsing after application of the electrodes to the subject's skin (in hours). It was shown that the resistance fell appreciably with time. With electrodes applied to the forearm, for example, and a measurement current of 30 μa, the value of the resistance was almost halved in the course of the first hour (Fig. 1, a). It might have been expected that for biocurrents these changes would be even greater, and also that the amplitude and frequency distortions would diminish in time.

We suggested a method of preparation of the skin, referred to as a combined method, which effectively lowered the interelectrode resistance at room temperature to 10-15 kiloohms. The skin beneath the electrode was gently treated with a paste consisting of soft soap and fine pumice (in proportions of 4:1 by weight), after which it was rubbed with ether, and then moistened with a conducting electrode paste whcih was used for recording the bioelectrical processes.

As criterion of the quality of the skin preparation, we used the ratio between the resistances measured by direct currents of 10 and 50 μa, which was equal to or less than 1.2.

With the combined method of skin treatment, neither amplitude nor frequency distortions, nor changes in these with time were shown on the recorded electrogram, since the value of the interelectrode resistance in this case was much smaller than the value of the input resistance of the amplifier.

After combined skin treatment, the value of the resultant interelectrode resistance was low (10-15 kiloohms) and altered insignificantly with time (Fig. 1, c).

The assumptions that amplitude and frequency distortions were present when electrograms were recorded from badly prepared areas of the subject's skin (treatment of the skin with alcohol) and that these decreased in time, thus were confirmed experimentally.

The electrograms were recorded from the subject's forearms. Electrode of the first recording channel were applied to well-prepared areas of the skin of the left and right forearms (interelectrode resistance less than 15 kiloohms). Electrodes coupled to the second recording channel were also applied to areas of skin situated alongside the first, and carefully prepared with alcohol. The electrical activity of the heart and of the abdominal muscles created biological potentials of equal amplitude and identical shape in the forearms if the skin beneath the electrodes were prepared by the combined method.

The second channel electrocardiograms, when recorded after various methods of skin preparation, had smaller values of the P, R and T waves and, as a rule, a larger S wave than the first channel ECG (Fig. 2, a). The shape of the T wave was also often distorted.

The decreased amplitude of the second channel ECG waves showed the presence of amplitude distortions. The unproportional decrease in the second channel ECG waves (the slower the wave, the more it was weakened) confirmed the presence of frequency distortions. The diminution of the second channel waves of the electromogram of the abdominal muscles, with a frequency of the order of 100-200 cps, as might have been expected, was insignificant. The assumed decrease in the distortions of the electrograms in the course of time after application of the electrodes to the skin of the subject was also confirmed experimentally (see Fig. 2).

Such gross distortions of the shape of the recorded electrical activity are inevitable when the forearm or leg is prepared with alcohol and when the value of the input resistance of the amplifier is between 0.5 and 1.0 meg.

From the electrograms recorded by the above method it is also possible to judge the resistance of the interelectrode circuit to the biocurrent. In one particular case, for instance, the value of the interelectrode resistance for oscillations of the type of the R wave of the electrocardiogram was 400 kiloohms, whereas for the T wave it was 1.3 meg.

The interelectrode (skin) resistance is not the same in different areas of the human body. We were able to show that a definite relationship exists between the value of the interelectrode resistance measured with a direct measuring current of the standard value of 10 μa – R_{10}, the ratio between the resistance measured with direct currents of 10 and 50 μa – R_{10}/R_{50} and the gradient of fall of the frequency characteristic of the interelec-

trode circuit. This relationship enabled the measurement of the interelectrode resistance to a direct current to be confined to a value of 10 μa for the measuring current. If this value was high, it followed that the ratio R_{10}/R_{50} was considerably greater than unity and the relationship between the interelectrode resistance and the frequency of the measuring current was clearly expressed.

The results obtained are of great importance to the electrographic method when applied in various conditions, including clinically.

SUMMARY

The author presents experimental material demonstrating that skin resistance in electrographic investigations plays a much greater role than has been considered hitherto.

It is shown that with the accepted method of preliminary skin treatment with alcohol or with a mixture of alcohol and ether, the recording of electrograms, especially those from the extremities, is subject to amplitudinal and frequency distortions and to the variation of these distortions in time, when prolonged application of the electrodes to the skin of the patient is practiced.

The author suggests a standard method of measuring the interelectrode resistance, another one providing for skin treatment eliminating electrogram distortions and a criterion of the quality of skin treatment.

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A MODIFICATION OF THE METHOD OF IMMUNOELECTROPHORESIS OF THE SERUM PROTEINS

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Grabar [1, 2] and Williams [4] described the so-called immunoelectrophoretic method of investigation of the serum proteins, which is a combination of electrophoretic fractionation in a solid medium and the Ouchterlony immunological precipitation method [3], in which two antigens diffuse from two points in agar, meet antibodies, and are precipitated in the form of lines. During investigation of human serum by the use of this method, Grabar found 19 precipitation bands in the form of arcs, corresponding to 19 antigens in the serum (and this is, therefore, the number of individual proteins).

We have slightly modified the technique of this method in order to secure greater standardization of the experimental conditions.

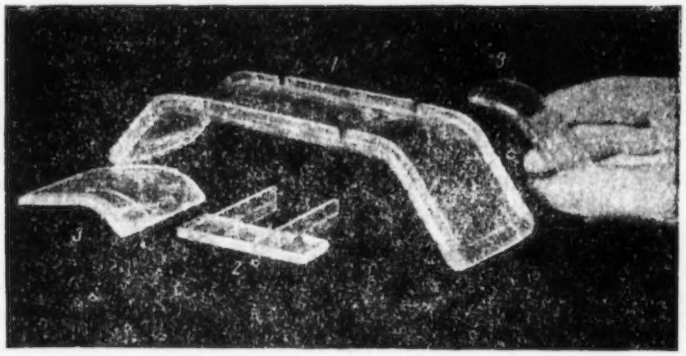

Fig. 1. Bridge-shaped dish for electrophoresis (1) with covers (3) and template (2) for the starting wells and the gutters. (The template is shown inverted).

Analysis of the immunoelectrophoregrams obtained by Grabar's original method shows that, even with slight changes in the conditions of electrophoresis, the picture is quite considerably changed. The arcs are formed in other places — nearer to the gutter with the antiserum or further away from it; their curvature is also altered.

On the basis of our experience we can say that the main conditions to be satisfied especially strictly are: 1) constancy of the distance from the starting well to the gutter filled with antiserum (an error of a fraction of a millimeter in a total distance of 4-6 mm is sufficient to alter the position and curvature of the arcs); 2) the gutter must be parallel to the long axis of the agar plate; 3) the volume of serum undergoing electrophoresis must be constant; 4) the shape of the starting well must be correct. Changes in the thickness of the agar layer also affect the electrical parameters of the experiment.

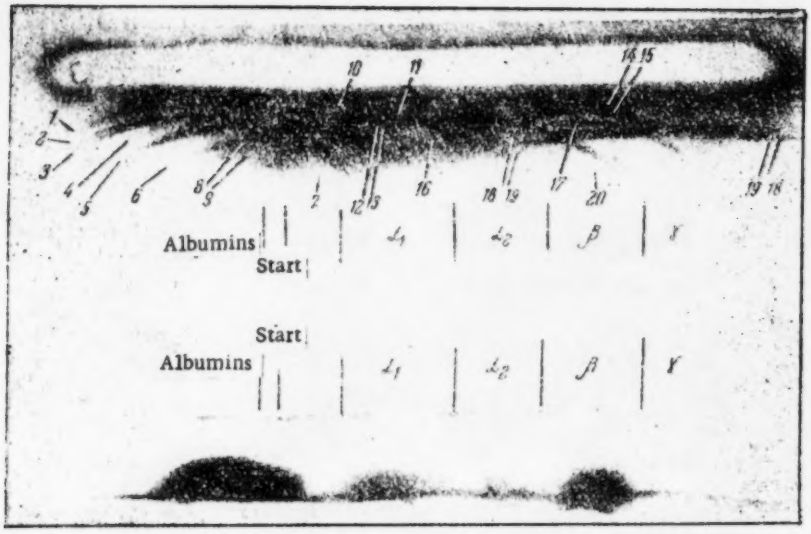

Fig. 2. An immunoelectrophoregram. 1-9) arcs in the albumin region; 10, 11) arcs in the α-globulin region; 12, 13, 16) arcs common to α_1- and α_2-globulins; 14, 15, 17) arcs common to α_2- and β-globulins; 18, 19) arcs of γ-globulins, going beyond the region of α_2- and β-globulins; 20) weak and inconstant line (lower part of the figure is the control half of the electrophoregram, stained without immunological development).

In order to secure standardization of the experimental conditions, we introduced a new detail into practice —the bridge-shaped dish. The glass slide with the agar film was not placed on the edge of the electrode dishes, as was described in the papers by Grabar, but on the bottom of a special dish (Fig. 1) which was shaped like a bridgewith side walls. The width of of the inner arc of the bridge between the walls was twice the width of a glass slide. The side walls were 4 mm high. The total length of the bridge-shaped dish, inclusing the curved legs which were made to stand in electrode dishes, was 24 cm. Two parallel glass slides were placed in the dish, and agar was then poured in. To prevent the agar from flowing down the legs at each side, covers were applied to the latter (see Fig. 1), fitting snugly against the side walls of the legs. The covers were additionally pressed against the side walls of the legs of the bridge by rubber ballons placed over them.

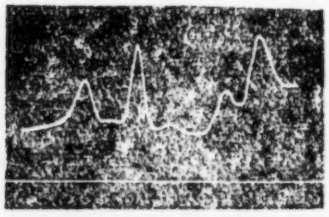

Fig. 3. Cross densitogram of the first 5 arcs in the albumin region, recorded on the MF-4 recording microphotometer.

A special template was placed over the glass side during its immersion in agar, to give the shape of the 2 future gutters and 2 starting wells (see Fig. 1). The starting wells, into which a mixture of serum and agar was later to be poured, could be cylindrical in shape (diameter 2 mm) or square in cross section. The patterns for the gutters for the antiserum were situated on both sides of the starting wells, each at a distance of 6 mm from the pattern for the corresponding well. The patterns of the gutters were strictly parallel to each other; the width of the gutter pattern was 3 mm. All four details were fixed to a connecting piece. The template was so designed that the patterns of the gutters were strictly parallel to the midline of the bridge-shaped dish; this was acrieved by the coincidence of special marking on the body of the dish and on the template.

After the agar had solidified, the template was removed. Correctly shaped starting wells for the serum and gutters for the antiserum remained in the agar. Next into wells was poured a mixture of serum with molten agar at 37° in proportions of 1:3. Excess was poured, in, so that the surface was raised obove the surrounding level. After the agar had solidified, all the excess lying above the general level was cut away with a razor blade

applied along side walls of the bridge. An agar plate of standard thickness, with a perfectly smooth and flat surface, was obtained. The height of the column of mixture of serum and agar was also predetermined. The covers were then removed from the legs of the bridge, and the legs were then placed, together with the overflow of agar attached to them, in electrode dishes for carrying out the electrophoresis.

At the completion of the electrophoresis ($2\frac{1}{2}$ -3 hours at 120 v) the layer of agar on the glass slide was cut away from the general mass around the edge of the slide, and the slide with its attached agar was removed from the bridge. Antiserum was poured into the gutters and the slides were placed in a humid chamber for 2 days. The advantage of this short time of development, which was possible only with agar plates of small size, was that it obviated the necessity of adding antiseptics, for colonies of microorganisms would not grow in 2 days at room temperature. In our experiments to investigate rat serum, 19 arcs of immunological precipitate appeared during the time of development (Fig. 2).

Next, in order to ensure complete removal of the soluble proteins, the slide was washed with physiological saline for not less than 6-7 days. In contrast to Grabar [2], we stained the agar plate with acid blue-black without preliminary drying. The preparations were also photographed undried, for drying deforms the agar slightly and its outlines become less sharp. Ill-defined, pale arcs shown up well by cross densitography on the MF-4 recording microphotometer (Fig. 3); the quantitative distribution of the immunological precipitate in the arc along an axis perpendicular to the axis of electrophoresis can also be ascertained from these densitograms.

We shall now consider a few methods of analysis of the arcs. It is sometimes important to detect very fine differences in the shape and situation of the arcs, since changes in the shape of the arcs indicate changes in the coefficient of diffusion of the corresponding serum protein, or a change in its immune properties which alters the proportions of antigen and antibody necessary for the formation of a precipitate, or, finally, changes in the amount of antigen protein. The most accurate comparison of the shape of the arcs can be obtained by the application method consisting of the juxtaposition of diapositives taken of the two preparations to be compared. This procedure, however, slightly complicates the picture, and it may be difficult to distinguish which arcs belong to which preparation. Comparison is simplified by staining one of the diapositives by the usual methods of toning as used in photography.

In some cases it is desirable to remove the image of certain arcs from the diapositive, which hamper examination. This is easily done by the use of a fine water-color brush, moistened with ammonium thiocyanate and ferrocyanide. In addition, copies made by hand, in ink, may be compared. For this purpose unexposed and developed (transparent) photographic film is applied to a phtograph of the immunoelectrophoregram, and the individual arcs are traced on it with a pen. Each arc is copied on a separate film; this facilitates the subsequent comparison. The second method is far more accurate although, at first glance, it may seem to lack the objectivity of the first method.

In conslusion it may be mentioned that all the improvements described are very simple to carry out.

SUMMARY

A modification of Grabar's immunoelectrophoresis method is described. Electrophoresis is effected on slides placed in a special bridged cuvette which serves both to hold the agar poured over the slides and to carry out electrophoresis.

The amount of agar in the cuvette is somewhat in excess of the required. The overflowing agar is cut off with a razor sliding along specially arranged edges of the cuvette, thus, always ensuring a uniform thickness of the agar layer.

The first compartments for the introduction of the serum and the troughs for the antiserum are also strictly standard in size.

This is achieved with the aid of special patterns (templates) placed on the slides while the agar is poured. Their position is fixed by markers on the cuvette.

Immunoelectrophoregrams obtained by this method are characterized by their constancy.

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TRANSPLANTATION OF A DENERVATED GASTRIC POUCH INTO THE OMENTUM

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The study of the mechanisms of secretion of the gastric glands cells for the use of several operative methods, including some the isolated gastric pouch is totally denervated [1-3, 6]. For a long time the Heidenhain pouch has been used for this purpose. It has been abundantly and convincingly shown, however, that the Heidenhain pouch preserves its sympathetic innervation almost completely, and its parasympathetic supply in part [1, 6].

Attempts to achieve total denervation of the isolated gastric pouch have proceeded in two directions. In 1912, Bickel and Katsch [5] suggested their modification of the classic operation. After the formation of the isolated pouch on the greater curvature by Heidenhain's method, these authors divided all the nerves visible to the naked eye passing from the greater omentum. Nevertheless, the Bickel and Katsch pouch was not completely denervated.

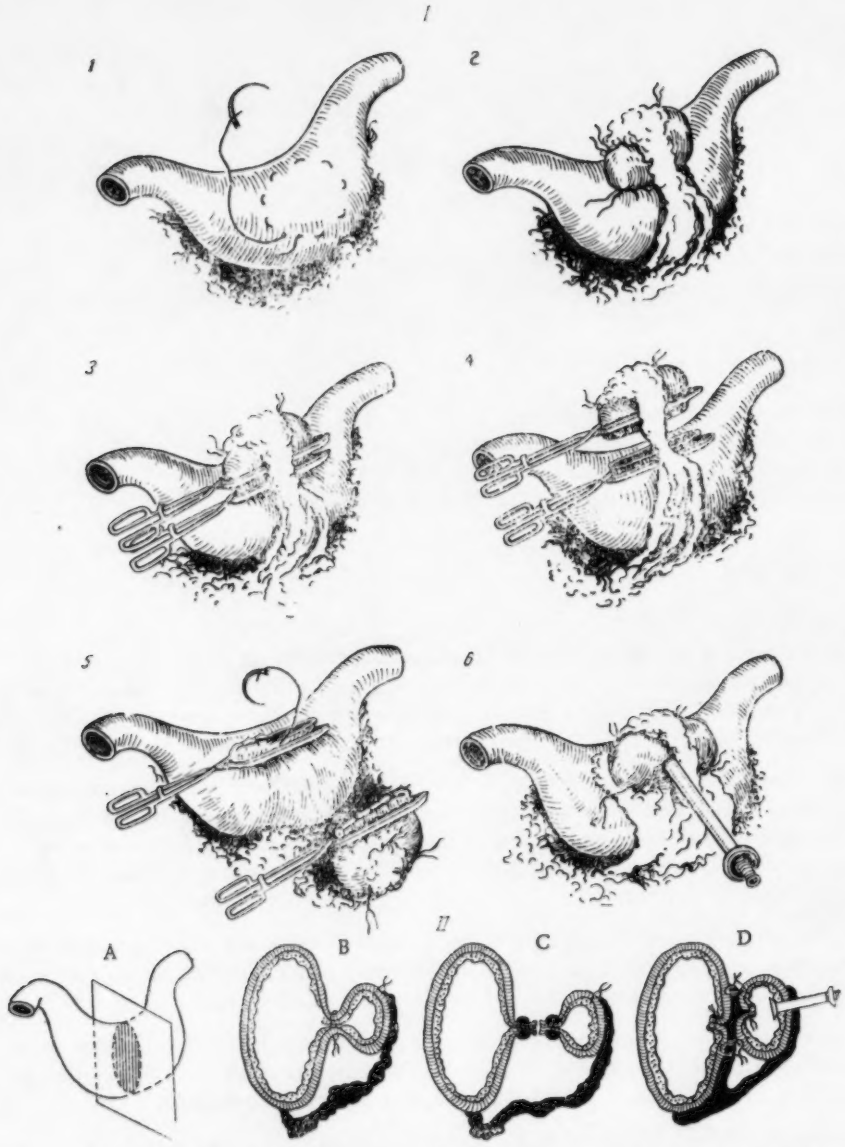
Ivy and his co-workers [8] used another method, the essence of which was that a portion of stomach receiving its blood supply from one or several major vessels was transplanted into the mammary gland of the dog. After a collateral circulation had become established, the neurovascular bundle was divided.

Gregory and Ivy [6] later suggested the subcutaneous transplantation of the fundal portion of the stomach, preserving the blood supply through the splenic vessels. Just as in the operations described earlier, the neurovascular bundle was divided 4-8 weeks after the establishment of a collateral circulation.

Grossman, Robertson and Ivy [7] increased the size of the transplanted area and transplanted the pyloric portion of the stomach.

Transplantation into the mammary gland has been widely used in the study of various organs, but the fundamental principle of the operation is not free from a number of serious defects. This does not mean simply that the method is technically difficult; only those organs and parts of organs which can be isolated and yet maintain an adequate blood supply can be transplanted into the mammary gland; furthermore, transplantation is possible only when the vascular bundle is sufficiently long and mobile; finally, during transplantation of part of the stomach, pancreas or loop of bowel beneath the skin, some degree of trauma to the organs is inevitable.

The transplantation into the omentum described in this article is largely free from these defects. We based our work on the numerous clinical observations of the use of the omentum for the creation of a collateral circulation.



Course of the operation (I) exterior view, II) schematic section). First stage of the operation (I, 1, 2 and II, B): 1) purse-string suture demarcating the transplanted area of stomach; 2) the purse-string suture is tied and the edge of the omentum is sutured to the transplanted area; B) the same moment of the operation in section. Second stage of the operation (I, 3-6 and II, C, D): 3) stomach clamps applied to the neck of the diverticulum; 4 and C) the pouch is resected between the clamps; 5) suture of the stomach and the pouch, transplanted to the omentum; 6 and D) sutures peritonized, gastric pouch fixed to the anterior wall of the residual stomach, and a fistula made into the pouch.

First stage of the operation. The portion of stomach to be transplanted is selected. If this area is in the anterior wall, a purse-string suture is applied to the serous and muscular layers. The approximate boundaries of the future pouch are shown in the figure.* The purse-string suture is loosely tied so that the cavity of the greater stomach communicated freely with the diverticulum thus formed. Incisions are then made in the serous mem-

* The gastric pouch thus formed is analogous to the pouch on the anterior wall with its nerve supply intact [4]. We take this opportunity to express our deep gratitude to our artist, V. N. Lebedev, for the drawings illustrating this paper.

brane on the surface of the diverticulum by 3-4 sutures. The first stage of the operation is now complete. The abdomen is closed in layers in the usual way.

Second stage of the operation. The development of a collateral circulation has usually reached the necessary stage 2-3 weeks after the first operation, and the next stage should be carried out at this time. After a month (or more), the vessels passing to the pouch from the omentum have become so large that the control of bleeding is made more difficult.

The technique of the second stage is as follows: two stomach clamps are applied to the neck of the diverticulum (see Figure) and an incision is made through all the layers between the clamps. Next, without removing the clamps, sutures are inserted into the stomach itself and the pouch. The sutured surface is peritonized with omentum. The pouch is then fixed to the anterior wall of the stomach by two or three sutures.

The operation is technically simple and its principle may be used in the transplantation of other abdominal organs.

SUMMARY

The author describes a 2-stage transplantation of a completely denervated isolated stomach pouch to the omentum.

This is a technically simple operation and gives almost the same results as the transplantation of organs into the mammary gland. It has, however, some advantage over the classical method.

The principle of this operation may be employed for transplantation of other abdominal organs.

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A CHRONIC BRONCHIAL FISTULA USING A POLYVINYL-ALCOHOL TUBE

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During the experimental investigation of various pathological conditions of the lung (atelectasis, acute and chronic bronchitis, bronchiectasis and so on) it is often necessary to make prolonged observations on the physiological processes developing in the lung. The existing methods for the experimental study of the pathology of the lungs are satisfactory for the solution of certain problems. These include, for example, the production of atelectasis in animals by obstruction of the bronchus by various foreign bodies [2, 3, 6] or the application of a ligature to the bronchus [1]; the production of fibrosis of the lung by ligation of the bronchial arteries [5] and of bronchitis, using various irritants for this purpose [4]. None of the classical methods, however, can be used for the functional investigation of the condition under study nor for continuous observations on a pathological process.

The most suitable method to be used for functional investigations of the lung in chronic experiments ought to be the formation of a chronic bronchial fistula. Attempts to use fistula tubes of metal, glass or rubber have, however, usually ended in failure, for sloughing has occurred at the site of suture of the tube and it has slipped out of the bronchus, and led to the development of pneumothorax and empyema. We have arrempted to form a bronchila fistula using a plastic tube (polyvinylalcohol) as used in thoracic surgery for the repair of large vessels and which, by being sufficiently hygroscopic, do not cause sloughing.

METHOD OF OPERATION*

In the preoperative period a pneumothorax was gradually induced in the dogs in order to prevent pleuropulmonary shock. The operation was performed under intratracheal ether-oxygen anesthesia, after preliminary injection of morphine and thiopentone. Throughout the operation the animals received antishock fluid, given by A. F. Fatin's drip method.

The bifurcation of the trachea was approached from the left doroslateral aspect, by resection of the third and fourth ribs. After procaine infiltration of the posterior mediastinum, the left main bronchus was isolated and a T-shaped fistula tube, with a central obturator end, was inserted into a longitudial incision in its membranous portion (Fig. 1). The tube was fixed into the bronchus by means of a silk purse-string suture, the ends of which were brought out in the paravertebral region and fixed to the skin. The operation wound was closed in layers. In the postoperative period penicillin solution was injected through the tube into the bronchus and intramuscularly for 7 days, and periodic drainage was undertaken. In three cases, when the external orifice of the tube was closed

* The operations on the dogs were performed with the active assistance of G. A. Myamlina, working in our laboratory.

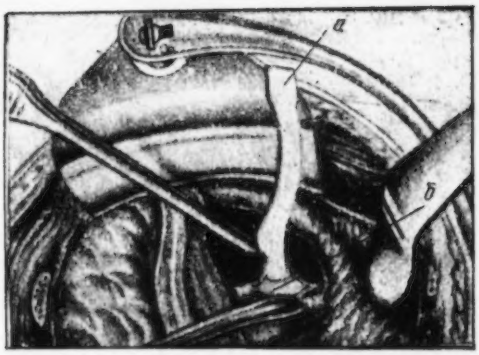

Fig. 1. Insertion of a polyvinylalcohol fistula tube (a) into the left main bronchus (b).

Fig. 2. Postmortem bronchogram of the dog Neron 39 days after operation. Cylindrical bronchiectases in the lobar and segmental bronchus.

Fig. 3. Dilatation of the lumen of the lobar bronchi (1, 2) in a gross specimen of the lung of the dogs Al'fa (on the 45th day after operation).

with a cork, an obturation atelectasis developed in the lung. Five dogs underwent operation.

From 35 to 45 days after operation, two of the experimental dogs, on which a preliminary vagotomy and stellate ganglionectomy had been done on one side, deeloped dilatation of the lobar and segmental bronchi, which was found at necropsy and in postmortem bronchograms (Figs. 2 and 3). Postmortem examination of all the dogs showed transpleural fistulae surrounding the plastic tube, in the form of a connective tissue tube. Under these circumstances the layers of pleura were smooth and shiny, with no signs of pathological change. The obstructed lungs were in a state of resorption atelectasis.

By the use of the method described it is possible, in the first place, by closure of the external orifice of the fistula tube, to make continuous observations on areas of atelectasis by the use of bronchography and bacteriological methods; secondly, to introduce into the ligated bronchus various chemical irritants, strains of microorganisms, drugs and so on; and thirdly, to carry out functional investigations (over a long period of time) on a lung isolated from the upper respiratory tract but retaining its nervous and humoral connections with the body as a whole.

SUMMARY

A surgical method of formation of a fistula of the main bronchus in dogs was performed by means of a polyvinyl alcohol T-tube. The simultaneous occlusion of the bronchus above the place of fistula formation isolated the lung from the upper respiratory tract.

However, gas exchange functions are maintained through the free external end of the fistula.

This method is of practical use in certain chronic experiments concerned with lung pathology. Bronchitis, atelectasis, bronchiectasis, etc. may be successfully studied in the course of their development by means of bronchography, bacteriology and administration of different substances and drugs through the open end of the fistula.

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THE USE OF A SIMPLE CONSTANT PRESSURE GASOMETER IN PHYSIOLOGICAL AND TOXICOLOGICAL EXPERIMENTS

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By the use of the apparatus described, in conjunction with a Marriott's jar, it is possible to obtain samples or a continuous flow of gas at a given constant pressure, greater or less than atmospheric pressure or equal to it. Such a constant pressure gasometer is very useful in the performance of many experiments:

1) in the determination of the volume of oxygen absorbed by an animal in experiments of long duration;

2) in the performance of prolonged exercises by animals by the static method in chambers of small volume, with maintenance of a constant concentration of oxygen in the chamber and measurement of the volume of oxygen absorbed by the animals;

3) in the introduction of gas into a chamber during the performance of dynamic tasks by animals;

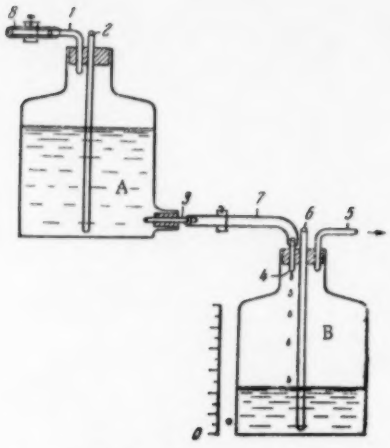
4) in the performance of experiments on the action of various concentrations of oxygen in a gaseous mixture at normal atmospheric pressure on animals;

5) in the storage of large volumes of gas so that samples may be taken from it at constant pressure.

The construction of the gasometer and the method of connecting its parts are shown in the illustration. The bottle A, with a delivery tube (capacity 1-20 liters, depending on the purpose of the experiment) is fitted with a cork, through which pass 2 tubes: one of these is short (1) and the other (2) reaches almost to the bottom of the bottle. A tube 3, inserted in the lower opening of this bottle, is connected by the rubber tube 7 to a short tube 4, which passes through the cork of a second bottle B. Tube 1 is also connected to a rubber tube 8, with a clamp. In the working position the clamp on tube 8 is closed, and the bottle A plays the role of a Marriott's jar. Besides the tube 4, the cork which closes the second bottle B also passes another short tube 5 and a long tube 6, reaching almost to the bottom of the bottle. Water from the vessel A enters through the short tube 4, and gas is supplied from the gasometer through the tube 5. Tubes 6 and 2 are used when charging the gasometer. The bottle B is calibrated, the divisions being marked (frombelow upwards) on a strip of paper glued to the bottle.

The bottles must be so arranged that their relative levels can be changed. In the working state, bottle A is filled with water and bottle B with gas. The pressure at which the gas is to be supplied from the gasometer is determined by the relative levels of the lower orifices of tube 2 in the bottle A and tube 4 in bottle B.

The bottles are arranged in this way when the gasometer is used for storage of gas, for maintenance of a constant concentration of oxygen in exercising chambers, or for determination of the volume of oxygen absorbed by animals. In the last two cases, soda lime or other absorption is placed in the chambers containing the animals, to absorb the carbon dioxide. In these conditions, the carbon dioxide exhaled by the animals is absorbed and does not affect the value of the pressure in the chamber. The consumption of oxygen leads to a fall in the pressure in-



Construction of the constant pressure gasometer and the method of assembly. For explanation, see text.

side the chamber, which causes oxygen to enter the chamber from the gasometer in volume equal to that used up. When the experiments performed require animals to be kept in a gaseous mixture with various concentrations of oxygen, the relative levels of the bottle A and B are the same as those described above (see Figure).

Apparatus based on the use of the Marriott's jar, for determination of the oxygen demand of animals kept in normal or altered concentrations of oxygen, have been used also in chambers described by other authors.

When gas has to be delivered from the gasometer into a chamber at an increased pressure, bottle A is placed at a correspondingly higher level than bottle B. If the gas should be supplied from the gasometer only at a given pressure below atmospheric, bottle A is placed below bottle B. The necessary difference in levels is easily calculated if it is remembered that the pressure of emission of gas from the gasometer (in cm of water) is equal to the difference between the levels of the lower orifices of tubes 2 and 4 (in cm).

The method described may also be used to supply gas to a chamber during exercise tests on animals by the dynamic method. In this case the bottle A is fixed above bottle B, and the tube 5 is controlled either by a screw clamp or by attachment of a cock. By changing the position of this cock or clamp, the volume of gas emitted from the gasometer may be regulated from zero to a given maximum value, which depends on the diameter of the tubes used in the gasometer.

Certain observations on the use of the gasometer may be given. When the gasometer is first charged, bottle B must be completely filled with water as a first step (best distilled), bottle A being empty. During filling of the gasometer with gas, the order of working is as follows. The clamp on tube 7 is closed and that on tube 8, attached to glass tube 1, is opened. The upper ends of tubes 6 and 2 are connected by a rubber tube (not shown in the figure). The appropriate gas is then pumped into the bottle B through tube 5 by means of a Komovskii (or other) pump; water is thus displaced into bottle A. When bottle B is full of gas the Komovskii pump is turned off (excess gas, at a slightly raised pressure during pumping, escapes from the gasometer), the rubber tube connecting tubes 2 and 6 is removed, and the clamp on tube 8 is closed. The clamp is then removed from tube 7 and the gasometer is ready for use. Other methods may be used for filling the gasometer.

When the gasometer is used for delivery of oxygen at atmospheric pressure or a slightly higher pressure, it is essential to make sure that no air bubbles are present in tube 4 and in the rubber tube connecting it to the delivery tube of bottle A, which would interfere with the working of the gasometer. They may be removed by sucking gas vigorously through tube 5.

SUMMARY

Employment of a simple constant pressure gasometer with the aid of a Marriott type of vessel is described. It enables a constant flow of gas to be obtained at a given pressure. It may be used in physiological and toxicological experiments.

A NEW METHOD OF INVESTIGATION OF VASCULAR PERMEABILITY

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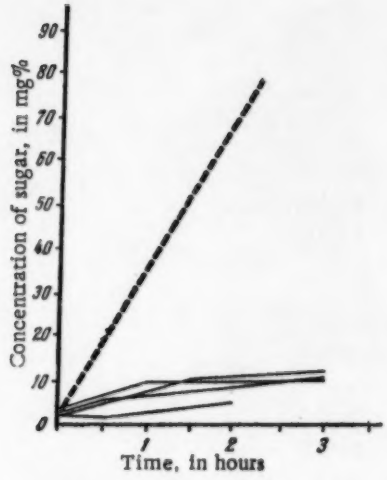
A widely used method of investigation of the vascular permeability is that of analysis of the contents of the closed natural cavities: the peritoneal cavity [2], the anterior chamber of the eye [3, 4], the cerebral ventricular system [1], and so on. The composition of the fluid which they contain is largely due to the character of filtration through the vascular wall. The degree of permeability of the walls of the blood vessels may be judged from the intensity of penetration into the cavity of substances injected into the blood stream, notably radioactive isotopes. This method has undoubted advantages over other methods of study of the permeability, in which the physiological state of the tissue must knowingly be disturbed (local inflammatory tests, for example). They do, however, have certain defects. The impossibility of estimating the volume of the cavity prevents quantitative interpretation of the results. The great physiological importance of the cavities restricts the use of the experimental procedures which can be used to alter the conditions of filtration. Such procedures include, for example, the creation of an artificial fluid in the cavity of known chemical composition and with given physical constants (osmotic and oncometric, etc.).

Following the suggestion of S. V. Andreev, a suitable artificial cavity for observations was created from an area of the urinary bladder in the rabbit, which was designed for investigation of the permeability of the vessels under chronic experimental conditions.

The operation of isolation of the area of bladder is as follows. On the abdominal wall, immediately above the pubic symphysis, a midline longitudinal incision is made through skin and peritoneum, 2.5-3 cm in length. The urinary bladder is brought out of the peritoneal cavity, the lateral vascular bundles are separated at the junction of the proximal and middle thirds of the bladder (for a distance of 5-7 mm) from its wall, and in the spaces thus formed a ligature is passed in a ring around the bladder. This ligature is then drawn tight, dividing the bladder into two parts. Those vessels to the organ lying above it are left intact. The proximal part of the bladder, in which are situated the orifices of the ureters and urethra, is buried in the peritoneal cavity, and the isolated distal part remains outside it. A suture is placed in the peritoneal incision, constricting the opening to a size which allows free passage of the stretched portion of bladder and the vascular bundles; a continuous skin suture is then inserted.

The operation is not complicated and trauma is slight, and the animals withstand the procedure well. The isolated portion of bladder, from which the urine is first removed and replaced by physiological saline, remains beneath the skin and serves as an artificial cavity. The histological structure of its wall undergoes no essential changes throughout the period of observation (8 months). At any moment the contents of the cavity may be taken for analysis through a special cannula or by puncture of the wall. They form a translucent, slightly opalescent fluid, containing protein (0.05-0.5 parts per 1000), a small number of leucocytes (1-5 per field of vision) and epithelial cells (not in every field). The volume and character of the contents remained unchanged for several months.



Curves of pentration of the blood sugar into a cavity filled with water (continuous lines) and with a 10% solution of ethyl alcohol (broken line).

By injecting various substances into the blood stream (dyes, sugar, radioactive isotopes, etc.) and estimating their concentration in samples taken from the cavity at various times after injection, a curve of penetration of the substance into the cavity from the blood stream is obtained. This curve shows the permeability of the vessels to the substances chosen. In most experiments we did not use foreign substances but estimated the penetration of the blood sugar into the cavity, which was filled with water. It will be seen from the figure that the curve of filtration of sugar is reasonable stable. The injection of substances altering the permeability of the vessels into the cavity has a marked effect on the character of the curve (see Figure).

The volume of the cavity (amounting in various animals to between 5 and 10 ml at atmospheric pressure) is known in each case, and its shape may be regarded as spherical, so that quantitative investigations may be carried out. For example, in assessing the degree of filtration, indices other than the changes in the concentration of the substance injected into the blood stream in unit volume of contents of the cavity may be taken. One such index is the total filtration in a given period of time, equal to Vk, where V is the volume of the cavity and k the final concentration of the substance in its contents.

A still more differentiated index is the filtration per unit area: $Vk/2\sqrt{4.5\ V^2\pi}$ in the example shown in the figure, for instance, the total filtration of sugar from the blood into the cavity in 2 hours was 1 mg, and the filtration per unit area 0.045 mg. When the cavity was filled with a 10% solution of ethyl alcohol instead of water, these indices were raised to 6.5 and 0.3 mg respectively.

If various substances are injected into the cavity, the changes in their concentration in successive samples taken from the cavity will give an indication of the filtration of the substances from the cavity into the blood stream.

SUMMARY

The author described a method of forming an isolated cavity from the distal portion of the urinary bladder in rabbits for investigating the vascular permeability in chronic experiments. The urinary bladder is ligated and thus subdivided into two portions. The distal part is led out under the skin. By analyzing the fluid samples obtained from the cavity at different periods after the administration of various substances into the blood or into the cavity, a curve is traced, depicting filtration of the substances from the blood into the cavity and vice versa.

By ascertaining the volume and the area of the cavity, it is possible to conduct quantitative investigations. Building up of artificial contents of a definite chemical composition and with known physical constants in the cavity enables the worker to vary at will the conditions under which the tested substances pass from the blood into the cavity and vice versa, and thereby to study the vascular permeability.

THE USE OF THE OXYHEMOMETER IN DETERMINING THE RATE OF THE BLOOD FLOW IN ANIMALS

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In order to determine the rate of the blood flow in animals we used a type 0-38 oxyhemometer and a solution of methylene blue.

Experiments were carried out on 12 rabbits and 8 cats. The pick-up of the oxyhemometer was applied to the rabbit's ear; into the auricular vein on the opposite side was injected 0.3-0.4 ml of a 2% solution of methylene blue. The moment of appearance of the dye in the vessels of ear with the pick-up was indicated by a fall in the photoelectric current. At this moment the oxyhemometer pointer is deflected to the right. The time from the beginning of the injection of the methylene blue solution to the beginning of the deflection of the oxyhemometer pointer (4 $\frac{1}{2}$-6 seconds) gives the rate of the blood flow. In cats under hexobarbital anesthesia, by injection of 0.3-0.4 ml of 2% methylene blue solution into the jugular vein exposed in the neck, the circulation time was 5-6 $\frac{1}{2}$ seconds.

When it enters the blood stream, methylene blue is diluted and quickly decolorized, as shown by the return of the oxyhemometer pointer to its initial position after 1-1 $\frac{1}{2}$ minutes. This enables repeated determinations of the circulation time to be made every 2-3 minutes, and in our experiments the results of these determinations varied within limits of ±0.2-0.3 seconds.

In experiments on white rabbits, because of the absence of skin pigment, in order to reduce the strength of the beam of light falling on the photoelectric cell, before adjusting the oxyhemometer it was necessary to interpose a filter (for example, a piece of white paper) between the photoelectric cell and the ear, and to leave it until the end of the experiment.

SUMMARY

The author attempted to determine the circulation time in cats and rabbits. For this purpose an oxyhemometer pick-up (type 0-38) was applied to the pinna and 0.3-0.4 ml of 2% methylene blue soltuion was injected intravenously (into the vein of the contralateral pinna in rabbits and into the dissected jugular vein in the cervical area in cats).

The time from the commencement of injections of methylene blue to the first deflection of the pointer of the oxyhemometer pick-up gave the circulation time and equaled 4.5 to 6 seconds in rabbits and 5 to 6.5 seconds in cats.

A NEW PLETHYSMOMETRIC APPARATUS (WITH AUTOMATIC ELECTRIC HEATING) FOR RUNNING MEASUREMENT OF THE ARTERIAL PRESSURE IN UNANESTHETIZED RATS BY A BLOODLESS METHOD *

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The apparatus which we have constructed differs from those suggested by other authors [5, 16, 17] firstly, by the presence of a group of plethysmometric measuring devices, enabling "running" determinations of the pressure to be made and shortening the time required for serial investigations; secondly, by a modification of the plethysmometer and, thirdly, by the method of local heating of the tail in the bath. Some authors [13] suggest using a Byrom and Wilson plethysmometric apparatus, with the addition of a photoelectric recording system to record both systolic and diastolic pressures. Besides the plethysmometric method, an oscilloscopic [6, 7], oscillographic [4], microphonic [8, 9] and other methods are described [3, 10-12].

PRINCIPLE OF THE PLETHYSMOMETRIC METHOD

The caudal vessels are compressed by a pneumatic cuff until the circulation is completely arrested. By releasing air from the cuff, the pressure within it is gradually reduced. When it becomes equal to the systolic pressure, blood begins to flow along the closed arteries into the peripheral part of the tail, which is placed in a plethysmometer. No blood escapes through the still compressed veins, so that the volume of the peripheral part of the tail begins to increase, causing the column of water in the capillary tube of the plethysmometer to rise. The start of this rise indicates the systolic arterial pressure, which is measured by a mercury manometer connected to the cuff.

We have shown that there are three main caudal arteries in rats (Fig. 1). The ventral (or median) caudal artery is the principal artery of the tail, and the largest in diameter, with a strong muscular layer, and it is the continuation of the median sacral artery; it is situated ventrally between the vertebral processes and is accompanied by two fine, variable branches. Two lateral caudal arteries, much smaller in diameter, are situated laterally and superficially; these are branches of the common iliac artery system. By using the compression cuff the pressure in the ventral caudal artery is usually determined, as it is largest in caliber, and therefore the one in which the pressure is slightly higher.

CONSTRUCTION OF THE APPARATUS

The apparatus consists of several (two or more, usually five) plethysmometric devices, mounted in a bath (Fig. 2, 1) with automatic electric heating to maintain a constant water temperature. Each measuring device consists (see Fig. 2 and Fig. 3) of a compression cuff, connected at the time of measurement of the pressure to an inflating balloon and a mercury manometer, and of a plethysmometer.

The compression cuff (Fig. 3) consists of a glass conical tube 1, through which is pulled a rubber tube 2

* A paper presented at the meeting of the Moscow Society of Pathophysiologists on September 24, 1958.

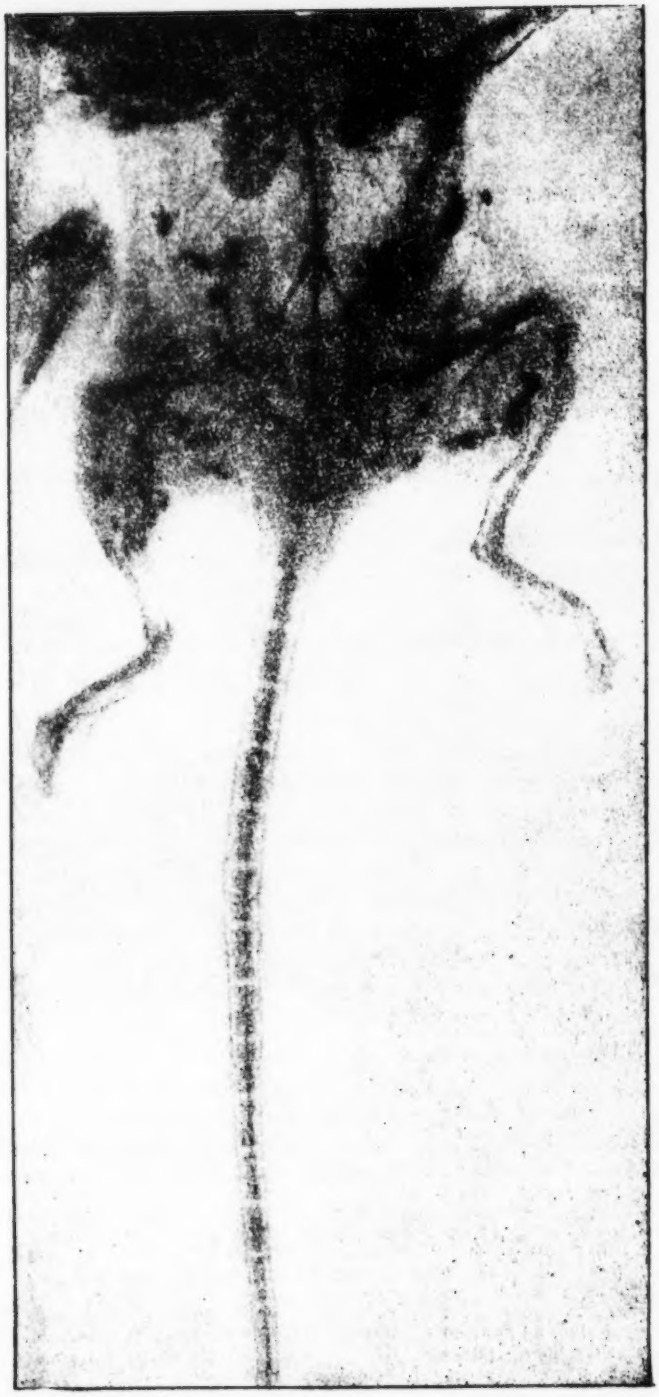

Fig. 1. The caudal arteries. Angiogram.

(made from a surgeon's glove), fixed by a thread to the everted edges of the glass tube; a side tube 3 is present, which is for connection to the inflating balloon and manometer. So that the rubber tube should not protrude through the rear opening of the compression cuff, which would cause a false rise of the column of water in the capillary tube of the plethysmometer, on this opening is placed a fixing ring 12, made of plexiglass or thin tin, fixed by threads 13 around the edges of the glass tube.

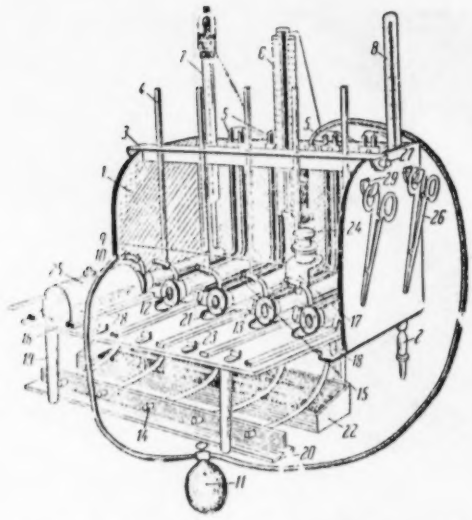

Fig. 2. General view of the apparatus for automatic electric heating. For explanation, see text.

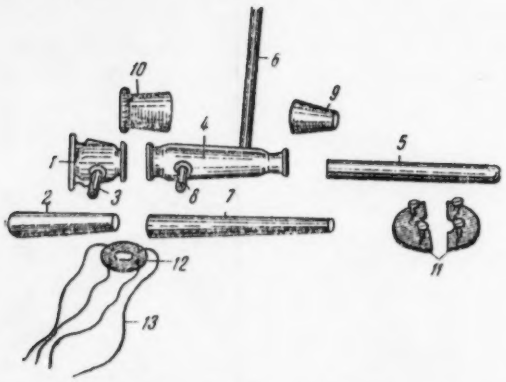

Fig. 3. Plethysmometric measuring apparatus in exploded view. For explanation, see text.

The dimensions of the glass tube are as follows: length 10-25 mm, diameter of the inlet 13 mm, diameter of the outlet 11.5 mm, internal diameter of the cylinder 18 mm, length of the side tube 3, 30-70 mm, its diameter 2-3 mm. The rubber tube has the same shape and size as the glass tube.

The minimum pressure necessary for complete closure of the inner edges of the rubber tube must not exceed 5-7 mm Hg.

The plethysmometer (see Fig. 3) consists of a working part 4 and an end tube 5. The working part consists of the conical glass tube 4 (corresponding to the shape of the tail) with a capillary tube 6, through which is drawn the rubber tube 7, made of fine surgeon's glove or from preserving rubber. The edges of the rubber tube are fixed by thread to the everted edges of the glass tube, as a result of which the closed cavity of the plethysmometer is formed between them (communicating with the capillary tube), which is filled with warm water during the experiment. The capillary tube of the plethysmometer 6 is fused in near the outlet (10-15 mm from its edge) and is directly vertically upward. The tube 8, fused at a distance of 10-15 mm from the edge of the inlet, is used for filling the plethysmometer with water and for draining it. In order to prevent water from the bath from entering the plethysmometers when they are placed in it, their rear portion are connected by thick rubber tubes 9 to end tubes 5 (Widal tubes are suitable).

In our experiments we used plethysmometers of different sizes, depending on the dimensions of the rats' tails. We give the dimensions of one of the plethysmometers which we most frequently used: length of the glass tube (see Fig. 3) 80 mm, diameter of the inlet 11 mm, diameter of the outlet, 8 mm, largest internal diameter 14 mm, diameter of the capillary tube 0.5-1 mm, length of projecting tube 8 from 25 to 30 mm and its diameter 2-3 mm. The dimensions of the rubber tube 7 corresponded to those of the glass tube.

The bath (see Fig. 2), designed for providing a constant temperature for the plethysmometric apparatus, consists of a rectangular box 1, on 4 legs, with the front cut out (for convenience in observing the plethysmometers, the manometer 6, the electrical contracts 7 and the mercury thermometers 8). The two hind legs 2 have screw adjustments with a maximum height of 120 mm, by means of which the rear portion of the apparatus can be slightly raised, which is essential when filling the plethysmometers with water, in order to expel air from them. A movable bar 3, through the holes in which pass the upper ends of the capillary tubes 4, and which can be firmly clamped to the upper edges of the sides of the box by the screws 27, serves the same purpose. The height of the front legs is 90 mm. For 5 plethysmometers the dimensions of the bath are as follows: length 450 mm, width 235 mm, height 240 mm. The bath is fitted with 4 heating coils, drawn through the glass tubes 5, which are filled with sand; they are situated between the plethysmometers. The coils are heated by the main supply (127 v). The temperature is regulated automatically by means of an electrical contact thermometer 7, included in the circuit to the coils through a relay. The mercury thermometer 8 is also provided for checking the water temperature.

In the front wall of the bath, near the top, the holes 9 are made (diameter 7 mm) for the short rubber connecting tubes 10, leading from the compression cuffs to the inflating balloon 11. In the front wall there are also holes 12 (diameter 15 mm) for introducing the rats' tails into the plethysmometric apparatus (distance from the lower edge of the hole to the bottom of the bath 20 mm). In the bottom of the bath are the special holes 13, into which the lower side tubes of the plethysmometers (see Fig. 3, 8), with their enveloping rubber tubes 14, through which the plethysmometers are filled with water, fit snugly. The free ends of these tubes fit in holes in the frame 20, fixed to the legs of the platform 16. In the center of the bottom of the bath is a hole with a metal tube 15 (diameter 15 mm) for emptying out the water.

The platform (see Fig. 2, 16), on which the chambers 25 containing the rats stand during the experiment, is removable and its dimensions are 450 x 220 mm; it is attached to the bath by the pins 18, and in front it is supported by two legs 19, 110 mm high. To the platform are fixed flat strips 28 (6 strips for 5 plethysmometers), 3-5 mm thick and 30-35 mm wide, between which the chambers for the rats are placed. Near the front edge of the platform, between each pair of strips, is a movable stop 23 for fixing the chambers with the rats. Semi-elliptical holes 21 are cut in the platform to allow escape of the feces and urine of the rats into the receiver 22, consisting of a rectangular plexiglass through, 450 mm long, 60 mm wide and 15 mm high.

Assembly of the apparatus. The compression cuffs, plethysmometers (see Fig. 3) and bath (see Fig. 2) are made ready beforehand. The plethysmometers and cuffs are tested for airtightness. The cuffs are fixed to the front of the bath near the holes for the animals' tails by means of two semicircular pieces of plexiglass (flanges) and 4 screws (see Fig. 3, 11). Next, by their lower side tubes, enclosed in rubber tubes 200-300 mm long, the plethysmometers are fitted into the special holes in the bottom of the bath (see Fig. 2, 13). The front portion of each plethysmometer is connected to the rear portion of the corresponding compression cuff by means of a wide rubber tube (part of a rubber teat) previously firmly fixed on to it. The end of this rubber tube is further secured to the compression cuff by means of a thread. In this way the internal cavities of the compression cuffs and plethysmometers are insulated from the water in the bath.

The upper ends of the capillary tubes are passed through the holes in the upper mobile bar of the bath (see Fig. 2, 3). This is fixed by the screws 27 on to the upper free edges of the sides of the bath in the required position. The platform for the animal chambers is placed in position by the front of the bath and fitted over the pins.

Temperature and duration of heating the animals' tails before measurement of the blood pressure. The minimum temperature in the measuring apparatus suitable for measurement of the blood pressure of intact rats with experimental renal hypertension, according to our preliminary findings, is 36-37°. At this temperature precise determination of the blood pressure, giving stable figures, is possible as a rule in intact rats after 5-15 minutes, and in rats with experimental renal hypertension from 10-30 minutes after the commencement of heating the tail (the rise in the level of the water in the capillary tube reaches 15-30 mm). The blood pressure varies little in the course of an hour (and sometimes longer) at this temperature, so that for standardization of the conditions of the experiment the selection of one single constant time of heating the intact rats and the rats with hypertension is recommended (in our experiments 20-30 minutes).

Measurement of the blood pressure. Measurements may be carried out on 4-5 rats at once. It is advisable at first to accustom the rats to sitting at rest in the chamber. The animals' tails are taken as far as the root covered with hair, and placed in the measuring apparatus, the chambers for the animals being placed on the platform. The chambers are anchored by the movable stops. The temperature of the water in the bath is raised to 37° by the electric heater. The plethysmometers are filled with water (37°) from the bath by means of a syringe, and clamps are placed on the rubber tubes (see Fig. 2, 14), best close to the bottom of the bath. After 20-30 minutes the measurement of the blood pressure begins. Three or four measurements are made on each rat in the course of 4-5 minutes and the limits of variation and the mean values are recorded. We may mention some sources of error: 1) false elevation of the column of water in the capillary tube of the plethysmometer due to movement of the animal; 2) pain on account of incorrect positioning of the rat in the chamber; 3) Incorrect placing of the rat's tail in the measuring apparatus (kinks, incomplete insertion); 4) measurement of the pressure in some rats without preliminary habituation to sitting in the chamber; 5) air pockets in the plethysmometer.

Systolic arterial pressure in normal and hypertensive rats. The systolic arterial pressure in normal rats, as measured by the apparatus just described, is as a rule between 70 and 110 mm Hg. In a few intact rats it may be higher (140 mm) or lower (65 mm). In experimental renal hypertension, resulting from the application of a cello-

phane capsule to both kidneys by our modified method [2], an increase in the blood pressure by 40-120 mm Hg was observed. The variation in pressure in the course of one experiment on intact rats was as a rule 10 mm or less, and only comparatively rarely was it 15-20 mm; in rats with renal hypertension it was usually below 20 mm. Day to day variations in the blood pressure of intact rats and in rats with hypertension (in the stage of stabilization wer usually 15 ± 5 mm. Quite rarely the day to day variations in intact rats were as much as 35 mm. Comparison of our figures for the normal systolic pressure in the main (ventral) caudal artery in rats with the figure obtained by the direct method in the femoral and carotid arteries by various authors [5, 13, 15, 16] showed that in many cases they were in agreement, and in a few cases they were slightly lower.

SUMMARY

A new plethysmometric apparatus with automatic local heating has been designed for conveyor (running) determination of the blood pressure in the caudal artery (ventral) in rats by the bloodless method. The apparatus differs from those already in use by:

1) the presence of a group of plethysmometric measuring instruments, which permits running determination of the blood pressure and reduces the time spent on serial work.

2) certain modifications of the plethysmometers,

3) the method of local warming, with the aid of a bath, of the tail. The temperature in the measuring instruments is 36-37° C and the duration of preliminary heating - 20 to 30 minutes.

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THE USE OF BF-2 GLUE FOR VARNISHING SMOKED PAPER KYMOGRAMS

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Kymograms varnished with an alcoholic solution of white resin very easily stick together when stored, become brittle and easily spoiled. Lowering the concentration of resin in the varnish reduces the stickiness, but greatly detracts from the quality of the varnishing.

We have had good results from the use of a 10% alcoholic solution of BF-2 glue (300 ml of alcohol to one tube of glue) for varnishing smoked paper kymograms. When rubbed with cotten wool, the varnished kymograms acquire a velvet sheen and remain in good condition during storage.

DETERMINATION OF THE pH OF LIVING TISSUES

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(Received May 17, 1958. Presented by Active Member AMN SSSR S. E. Severin)

The determination of the active hydrogen ion concentration of living tissue has been extensively used in solving certain problems in normal and pathological physiology. This index has acquired particular importance in pathology in connection with the study of the nature of the inflammatory reaction, as in H. Schade's [8] research. In spite of the fact that Schade's theory has been subjected to very serious and well-grounded criticism, the determination of the pH of inflammatory tissue is still a problem of the utmost urgency. No accurate and reliable methods of determination of the pH of the tissues of a living animal, exist yet, however.

In the research of Schade and his co-workers [13] the pH was determined by means of a hydrogen electrode. In this method, the hydrogen ion concentration is not measured directly in the tissues, but in the tissue fluid which is drawn from the tissue into the tip of the electrode. Because of this, the method has been found unsuitable for the determination of the pH of comparatively dry tissues as, for example, the normal uninflamed skin.

This last aspect is stressed by A. D. Ado [1, 2], who used this method to determine the pH of animal tissue in inflammation of allergic origin or due to turpentine. A fundamental defect of the hydrogen electrode is the very slow development of the potential (40 minutes to 2 hours). In such conditions it is nearly impossible to study the pH changes during the initial stages of development of inflammation. It should be added that the platinum black gradually comes away from the electrode when it is buried in the tissues; this may interfere with the working of the electrode. The method is very clumsy and requires a complicated apparatus for the continuous supply of hydrogen.

The theoretical importance of a solution of this problem necessitated a search for more adequate methods of estimation of the pH of living tissues. This provided the impetus for the development of various modifications of the hydrogen electrode and for the introduction of new methods. Nevertheless it has proved almost impossible to overcome the technical difficulties in measurement of the pH in vivo.

Lanz and Malyoth suggested a gold-hydrogen-iridium electrode, which had a number of advantages. However the variations in potential by this method were so great that, in order to obtain reliable data, it was necessary to take the mean values of a large number of successive measurements [11].

Many workers, among them A. F. Gol'dberg [4] and A. O. Voinar [3], used a quinhydrone electrode introduced directly into the tissues for determination of the pH. Subsequent verifying investigations showed that this method also was unsuitable. V. P. Grechanovskii and I. I. Matusis [5], in particular, showed that when the quinhydrone electrode was used the potential changed so quickly that it was difficult to settle on any particular figure. Another grave defect of the quinhydrone electrode is its irremediable error with regard to protein and salt. There is no guarantee that, when the electrode is inserted, sufficient quinhydrone enters the tissues to cause saturation.

In just the same way, the antimony electrode [9] and the platinum electrode coated with MnO_2 [10] and others failed to justify themselves in the measurement of the pH of living tissues.

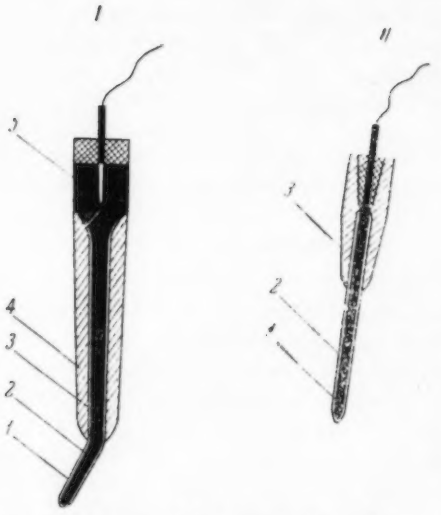

Fig. 1. Scheme of the constructions of the electrodes. I) Glass microelectrode, filled with mercury: 1) glass capillary tube; 2) mercury; 3) 0.1 N HCl solution; 4) mixture of resin and paraffin wax; 5) widened part of the body of the electrode with the wire immersed in mercury; II) glass electrode with platinum base: 1) platinum needle; 2) glass film; 3) mixture of resin and paraffin wax.

The best prospects for the determination of the pH of the tissues are given by the glass electrode, for it does not require the introduction of additional substances into the tissue and is not harmed by oxidizing and reducing substances in the tissue; its potential is quickly established. Attention was first drawn to this by Voegthlin and his co-workers [14], who suggested and used their variant of the glass capillary electrode in their experiments. With many undoubted advantages over the methods described above, this electrode has, nevertheless, certain defects – it is very fragile and has a high internal resistance; it is also necessary to use two additional semielectrodes of calomel.

We found no reports in the Soviet literature of investigations of the pH in vivo with a glass electrode.

The aim of our research was to study the pH of the tissues of a living animal in various pathological processes. Taking into consideration the defects of all the known methods used for this purpose, and also the fact that, in general, this problem is associated with technical difficulties, we started our work by attempting to produce a model electrode, free as far as possible from the defects inherent in the electrodes described above.

EXPERIMENTAL METHOD AND RESULTS

We prepared two types of glass electrode; their principle of action did not differ in its general outline from that of other electrodes of this type (this question is discussed in detail in the exhaustive monograph by V. A. Pchelin [6]).

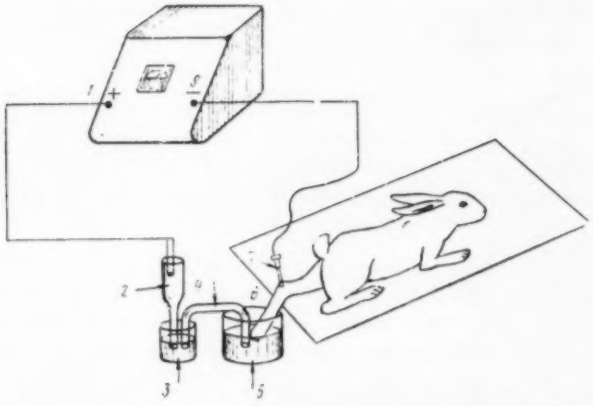

Fig. 2. Scheme of the measurement circuit. 1) Apparatus (positive terminal); 2) calomel semielectrode; 3) vessel containing a saturated solution of potassium chloride; 4) electrolytic bridge; 5) vessel containing physiological saline; 6) hind limb of a rabbit; 7) glass electrode buried under the skin; 8) negative terminal of the apparatus.

The first electrode consists of a platinum needle (Fig. II, 1) covered with a thin film of glass with a high sodium and magnesium content (É-S 2 glass). At the junction of glass and metal a reasonably stable potential is formed. The main conditions for the proper working of this electrode are an absolutely close covering of the metal with glass, without any space or air bubble, and very strict insulation of its outer surface (in contact with the test solution or tissue) from the lead connecting the platinum wire to the apparatus. The outer surface of the electrode above the buried portion (i. e., the strictly working portion) is coated with a mixture of resin and paraffin wax. Firstly, this produces a working surface of the electrode of strictly constant size, and secondly, it prevents the formation of a hygroscopic film on the glass surface, and leakage of the current. After it has been made, the electrode must be immersed for a week in a 0.1 N solution of hydrochloric acid and then for not less than 2 weeks in distilled water. In the intervals between investigations the electrode must be kept in distilled water, for drying leads to a deterioration in the electrode properties of the glass.

When properly made, the electrode gives a stable and rapidly established (in 20-30 seconds) potential, and is very convenient to use – with a long enough wire it can be taken to an animal in any position. The necessity for a second calomel semielectrode is obviated, but the considerable length of the working part of the electrode (1. 5-2 cm) introduces difficulties into the work. In the second type of electrode we were able to shorten the working part. This electrode (see Fig. I, 1) consists of a capillary tube – the working part proper – , 9-10 cm long, and a body, widened at the top and sealed with an airtight cork. Through the cork passes a copper wire, one end of which is connected to a measuring apparatus and the other is immersed in the mercury which fills the whole electrode (in the manner of the macroelectrodes suggested by A. I. Rabinovich and V. A. Kargin [7]). In order to stabilize the potential at the mercury – glass junction, the electrode is at first filled with normal hydrochloric acid, and the acid is then gradually replaced by mercury. A thin film of hydrochloric acid is formed between the liquid mercury and the glass, thus ensuring constancy of the potential inside the electrode.

Immersion of the electrode and coating of its body with the mixture of resin and paraffin wax are done just as for the first type of electrode. In its construction, the necessity for using a second calomel semielectrode is obviated. In this electrode, the potential is established rather more slowly than in that described above (1-1½ minutes), but it is more stable. Before each experiment the electrodes must be calibrated in 2-3 buffer solutions of known pH. The internal resistance of both electrodes was high, but did not exceed the input resistance (100 meg) of the "RFT, TUR-190 pH-meter", with which the measurements were made.

When the measurement circuit is being assembled the following points are essential: 1) the glass electrode must be connected to the negative terminal of the apparatus and the calomel semielectrode to the positive terminal; 2) the lead from the electrodes, the terminal and the apparatus itself must be carefully screened; 3) all parts of the measuring circuit must be insulated from each other, for the potential is so small that the least leakage of current may cause essential distortion of the experimental results.

Experiments were carried out on rabbits, lightly anesthetized and fixed to an electrically insulated bench. The electrode, fixed in a special clamp, was introduced through a preliminary puncture wound into the part of the body selected for investigation (beneath the skin, into the muscle and so on). Care had to be taken to ensure that the puncture wound was not bleeding and that the thick portion of the electrode firmly prevented the access of air to the tissue.

The hair in the region of the talocrural joint of the rabbit was carefully shaved; the skin incision was then made, inevitably with bleeding, and the joint was immersed in the vessel containing physiological saline. This vessel connected through an electrolytic bridge to a vessel containing a saturated solution of KCl, into which was dipped the end of the calomel semielectrode. The scheme of the measuring circuit is shown in Fig. 2.

With accurate and delicate working, requiring the development of skill in certain directions, reasonably precise estimations of the pH of the tissue can be made.

Using this method, we determined the pH of healthy rabbits tissues. The subcutaneous cellular tissue and striated muscles were investigated. As a result we were able to show the hydrogen ion concentration was reasonably close in different rabbits. Under morphine anesthesia its average value was 7.27, with a mean square deviation of ±0.17. Individual variations in the pH of different rabbits lay within limits of 0.1-0.5 pH units.

The electrode may stay in the tissue for several hours and continue to show constant values of the pH; this enables accurate determinations of the pH to be made in the living animal under normal and pathological conditions at intervals over a period of time.

The characteristics of the pH changes in inflammatory tissues will be the subject of our next communication.

SUMMARY

Two types of glass electrodes for the electrometric determination of the pH of the tissues in the living body are suggested. The pH of the tissues of healthy rabbits and the pH shifts occurring in an inflammatory process were studied with the aid of these electrodes.

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